5-22-2011

Phenotypic Plasticity and Transgenerational Epigenetic Inheritance in Response to Varying Light Levels

Jack Michael Colicchio
Dickinson College

Follow this and additional works at: http://scholar.dickinson.edu/student_honors

Part of the Biology Commons

Recommended Citation

This Honors Thesis is brought to you for free and open access by Dickinson Scholar. It has been accepted for inclusion by an authorized administrator. For more information, please contact scholar@dickinson.edu.
Phenotypic Plasticity and Transgenerational Epigenetic Inheritance in Response to Varying Light Levels

Jack Colicchio
Dickinson College
May 2011

Submitted in Partial Fulfillment of the Requirements for Honors in Biology, May 2011

Approved by

Dr. Carol Loeffler, Supervisor
Dr. Kirsten Guss, Reader
Dr. Tom Arnold, Reader
Phenotypic Plasticity and Transgenerational Epigenetic Inheritance in Response to Varying Light Levels

The shade avoidance response and divergent populations of Solidago rugosa, and transgenerational epigenetic inheritance and signs of “Group Selection” in Arabidopsis thaliana

Jack Colicchio
May 10, 2011

ABSTRACT
Epigenetics provide organisms with the ability to alter gene expression, modifying the way two identical pieces of DNA can be expressed. These mechanisms may activate in response to various environmental conditions, allowing for different environmental conditions to differentially affect the gene expression of a given organism. If this change in gene expression results in different phenotypes we can describe this response as an example of phenotypic plasticity. Epigenetic mechanisms differ from traditional transcriptional regulators in that the modification can, at least in some cases, be passed along to the next generation. In theory this can allow for the transmission of acquired characteristics from one generation to the next. In both field measurements and greenhouse growths we found that Solidago rugosa grows to a higher height to width ratio in the shade than it does in full sun (Greenhouse: $F=44.03$, df=1,87, $p<0.0001$) (Field: $F=142.68$, df=1,116 $p<0.0001$). Working with Arabidopsis thaliana we found that the increased fitness due to high light levels may be transmittable to future generations. This was not shown to be quite statistically definitive ($F=2.89$, df=1,88 $p=0.0928$), and will be a good candidate for future research.
INTRODUCTION

Epigenetics can be defined as the study of any material outside of the underlying DNA sequence that can cause a heritable change in gene expression within a given cell or organism. While the ability to regulate gene expression based on environmental cues can partially be explained by simple ligand->receptor->transcription factor interactions, these pathways don’t offer any way for an animal or plant to make semi-permanent changes to their gene expression. However, recently there have been numerous other molecules besides the DNA itself that have been shown to affect the ability for a given section of genetic material to be transcribed. One of these methods by which an organism may control which sections of DNA will remain active and which will be silenced is through the cytosine base methylation. While the mechanisms that control the methylation of an organism’s genome remains largely a mystery, there are certain aspects of this process that make it a very interesting target for further studies. Methyl groups in a gene’s promoter region have been shown to significantly down-regulate the given gene, and at least one of the major proteins responsible for methylating a given segment of DNA(DNMT group proteins) has been identified (Law and Jacobsen, 2010).

Even more intriguing from an evolutionary standpoint is that the location of methyl groups on an organism’s genome can be passed not only through cell lines, but also at least partially from the germ line of one parent into the zygote of its offspring, allowing for the transmission of methyl patterning between generations(Bosdorf et al., 2008). This opens up the possibility for organisms to have the methylation patterning of their genome changed within their lifetime due to developmental or environmental cues, and then to pass on this new epigenetic marking to their offspring. Jablonka (2008) described the importance in recognizing the duality that epigenetic systems have in both determining developmental changes, and as an inheritance system. While the scale in which this actually occurs is still somewhat unknown, numerous recent findings have shown that it may play an important role in allowing for the “soft-inheritance” of traits that allow for plants with the same genetic material to appear different due to the environment they were raised in.

Transgenerational, environmentally induced epigentics is, at its core, dependent on phenotypic plasticity at the given loci. Phenotypic plasticity can be defined as the ability to produce different phenotypes in response to different environments (Schmalhausen, 1949). Transgenerational epigenetics allows for these changes in gene expression based on environment to be passed on to the next generation, even with the removal of the initial environmental stimuli.

The reason I chose to work with plants rather than animals in my research into epigenetic inheritance is two-fold. First, and most importantly, as sessile organisms, plants do not have the ability to move from where they fall to a more favorable environment. For this reason a plant located on an ecotone of a forest and a grassland would be forced to develop in whichever ecosystem it landed in, compared to an animal that could easily move to whichever environment better suited its individual phenotype. This ecotone example is a macro-scale example of an evolutionary system that probably most often works in response to much smaller-scale environmental variations in soil composition, water availability, sun exposure, or predator presence, but does provide an easy model by which to imagine its potential advantages. The
other reason that plants would be more advantageous to study is that they lack the ability that certain animals have to teach their offspring techniques to survive in their specific environment. While animals have developed the ability to teach their offspring through example special tricks that allow them to better survive in their environment, is it possible that certain plants have likewise developed the ability to pass down a "learned knowledge" of their environment to their offspring not through physical demonstration, but rather through a system of genetic imprinting? The fact that methylated sequences of DNA also show a higher rate of point mutation also interests me as in theory methylation could be used as a testing stage for a new genotype (Jablonka, 2009). If the methylation of a given promoter turns it off, and if this deactivation is an advantage, we should expect to see methylation continue at this locus. Since methylation increases the tendency toward genetic mutation we are now more likely to see a mutation leading to a long-term deactivation of this gene.

I am performing experiments on two separate plant species during my independent research. The first of these is *Arabidopsis thaliana*, the main plant model organism. Seeds were ordered from the Arabidopsis Biological Resource Center with both wildtype specimens and a mutant line homozygous recessive (loss of function mutations) in three genes (drml, drm2, cmt3) important for the production of key proteins in de novo methylation and the maintenance of non-CG methylation. Non-CG methylation refers to the methylation of a cytosine nucleotide that is not directly upstream of a guanine basepair. This type of methylation is found in mature plants and bacteria, but only in developing organisms in the other kingdoms (Jacobsen, 2002). It is important to also note that the proteins involved in non-CG methylation in plants is not found in any other type of organism. Another interesting thing about non-CG methylation is that it has been shown to have an impact on developmental abnormalities showing that it may be involved in controlling gene expression in a similar way to CG methylation. In fact, recent research has shown that in plants CG-methylation may have more of an impact on silencing transposons, while non-CG methylation may be the most important expression mediating form of methylation (Zhang et. al, 2006). Non-CG methylation has been shown to be developmentally important in plants, and interestingly we find that after demethylation the plant is able to effectively re-methylate the same areas of the genome that were methylated pre-treatment (Chan et al., 2006). By looking at the drml, drm2, cmt3 triple mutants as well as wildtype plants I am interested in seeing if certain epigenetic phenotypes are unable to be adopted and passed along without these genes functioning.

Minimal research has been done on the importance of this form of methylation, the mechanisms behind it, and the ability for these methylation patterns to be passed on between generations. The three environmental variables I began my research testing are predation, light levels, and nutrient levels, but by the end my focus switched completely to light levels. I measured first to see if there are any phenotypic changes produced by these various environmental conditions, and secondly if any of these phenotypic changes can be passed on between generations. Light levels are an ideal environmental variable to analyze when studying potential influencers on both phenotypic plasticity and transgenerational epigenetics. This was explained succinctly in Pigliucci and Kolodynska’s 2002 paper on plastic responses to light levels in *Arabidopsis thaliana*.
"Light is a fundamental heterogeneous environmental factor for plants, and different aspects of light availability (quantity, daylength, spectral quality, angle of incidence) are perceived by specialized photoreceptors (Ballare, 1999; Lasceve et al., 1999) that induce responses that are considered adaptive (Schmitt et al., 1999). For example, plasticity in leaf morphology induced by light quantity is one of the best-known responses to environmental heterogeneity (e.g., Nunez-Olivera et al., 1996)."

This passage discusses light as a key environmental variable in prompting phenotypic plasticity, but, in the case that these responses to light are dependent on changes in methylation state, we may find that they can also be transgenerationally inherited.

While there is large-scale research being done on the effect of various stressors on the epigenetic make-up of organisms, there is a dearth of information on the phenotypic effect that these changes have on the plant (Verhoeven et al., 2010). Much of the research done on variable methylation in plants has been done within the past few years, and in particular novel research by Koen Verhoeven and Christina Richards has contributed to the rapid growth of this field. Interesting to note is that some of the primary research on transgenerational epigenetics has been done with the Common Dandelion, Taraxacum officinale, which reproduces by apomixis (asexual reproduction) potentially changing the levels in which methylation patterns are reset (Verhoeven et al., 2010). By looking at differences in the growth patterns between plants whose parents were grown in different environments, but which are currently grown in common conditions, allows us to tell which types of phenotypic plasticity may be epigenetically inherited. The effect that a parent plant’s environment and growth rates have on their offspring’s ability to outcompete other individuals may be at least partially controlled by the epigenetic make-up of these plants. The evolutionary implications of environmentally induced, or at least altered, gene expression is great and may play an important role, along with standard developmental cues, in leading to altered gene expression between plants with similar genetics. By combining phenotypic-based analyses of phenotypic plasticity and transgenerational epigenetics with molecular studies into the methylation changes during experimental treatments we can begin to see how different environments cause a change in methyl patterning. This information will not only allow us to test what environments cause epigenetic changes, but also what genomic sequences are targeted by methylation state regulators under various conditions.

The second plant that I am working with, Solidago rugosa, allows me the opportunity to take advantage of a local plant growing in an environment that seems to have already laid the groundwork for a study on environmentally induced divergent epigenomes. At Reineman Sanctuary rough-stemmed goldenrod is found growing fairly widely, both in the open, clear cut field, as well as in the adjacent forest which has been allowed to regrow. These adjacent field and forest populations seem not separated enough, spatially or temporally, to allow for their genomes to become truly isolated. The fact that this plant also reproduces asexually allows us to take measurements on this plant’s growth patterns throughout numerous life cycles without having to worry about random genetic mixing. The main question I will be looking to answer with this plant is whether these environmentally induced differential phenotypes are passed on to
offspring living in a different environment, or if the epigenome is completely reset between generations (Law and Jacobsen, 2010). It was interesting to note if seedlings of parents in woods and parents in field habitat revert to a certain growth pattern, while the plants grown from previous year’s roots may maintain growth patterns typical of their environment. Because this species often lives on the edge of two vastly different environments, the ability for a parent plant to pass epigenetically stored environmental knowledge between generations could provide a plant with a competitive advantage. Another interesting variable is that when taken into the greenhouse we will not be making changes to the ratio of Far Red: Red light received by the plants, rather we will simply be effecting the total amount of light received through shade cloth. The “Shade Avoidance Response” is a set of growth patterns that are often seen when plants are placed in shade, but it has been stated that the ratio of Far Red: Red light is key to induce this response (Ballare et al., 1990). During this type of study it is important to take great care in determining which types of variation could be due to genetic factors, epigenetic factors, or simply environmental variation between sites. This must be achieved through comparing plastic responses within generation to responses shown in future generations, and must be treated very carefully on a case by case basis. While the mechanisms behind these plastic response are very important and must also be studied, analysis of which characteristics may be under epigenetic control, and to what extent they can be modified, is the focus of this paper.

MATERIALS AND METHODS

*Arabidopsis thaliana:*

I purchased seeds of two mutant seed stocks and one wild type gene stock. The *drm1 drm2 cmt3* mutant seed stocks were produced by Steve Jacobsen of Cold Spring Harbor Laboratory. As described in the introduction section, this line was selected because non-CG methylation may prove to be an important factor in flowering time in plants, and this mutant strain is lacking a properly functioning methylation pathway. The other mutant selected had a loss of function mutation for the DNMT protein. This mutant strain was selected because this protein is the primary protein involved in CG methylation in all organisms. As methylation is believed to be one of the primary methods of epigenetic inheritance, these mutant lines would provide us with information as to whether or not any findings are related to differential methylation. The DNMT mutant was dropped from experimentation due to an inability to successfully get it to flower in our lab conditions. The wild type plants selected were of the Columbia ecotype. These were selected because they are the plants that the mutant germline was bred from.

For the first generation of growth plants were grown in 3”x3” cells within seed flats, while later generations were grown in one inch square cells. All watering was done with distilled water, and after the second leaf pair developed the plants were fertilized weekly with *Miracle Gro* (24-8-16) all purpose fertilizer mixed in the ratio of 0.5 tablespoons per 2000 mL distilled water. Plants were watered from below throughout growth.
In order to insure accurate seeding, a method of planting was developed that involved picking up each seed individually with a micro-spatula, and then pipetting into the appropriate cell. After seeds were planted they were misted and then covered with saran wrap and placed into growth chambers. Once the majority of seeds had sprouted the saran wrap cover was removed. The growth chambers were set at 23°C with either 18 or 13 hours of light per 24-hour cycle depending on the growth conditions desired. Plants were rotated between the growth chambers to prevent any errors due to different chamber conditions. The majority of the methods used to grow *Arabidopsis thaliana* were adopted from the CSUCLA Vellanoweth Lab guide for growing Arabidopsis (Vellanoweth, 1997).

**EXPERIMENTAL PROCEDURES**

I worked with several different environmental variables to test the effect these variables have on plant growth and if these patterns could be passed on epigenetically. First, the purchased seeds were grown in a set of uniform conditions to help minimize the effect that variable growth environments in the past generation would have on the epigenome of the plants. In this phase of the experiment plants were grown at 18h and 13h of sunlight and at two separate fertilizer levels (0.5 and 1.5 tablespoons per 1000 mL). All three gene lines of Arabidopsis were grown during this cycle of growth. After this cycle I determined that the high fertilizer levels were a stressor to the Arabidopsis rather than simulating any natural variance in nutrient levels and dropped this treatment from the experiment. This was determined due to poor survival rates and overall plant health of the plants in the high fertilizer environment. Also, after talking to other scientists working with Arabidopsis I learned that that plants can thrive and reach maturity without any additional fertilizer. The 13h sunlight treatment slowed down the flowering cycle of the plants dramatically, but the plants did eventually flower and produce seeds. Other problems were encountered during this round of growth, including low flowering and high mortality rates by the DNMT mutants, leading to plants of this line being dropped from the experiment. The first semester held numerous setbacks, which although they were not unexpected, certainly did teach me that the best experiments on paper often become more challenging and time consuming in reality. While Arabidopsis is grown worldwide and there are countless resources on their growth, the ability to grow these plants under low light conditions, and to collect data and seeds from them on an individual level while attempting to limit cross pollination, forced me to develop new methodology. Planting these seeds was one major challenge as most methods involve planting a bunch of them in a cell through crude techniques. I, however, wanted to insure that only one seed was planted in each cell, requiring me to develop a new methodology involving spreading out the seeds on a Petri dish before individually selecting seeds with a microspatula and planting them on the soil.

With the seeds collected I began a variety of new experiments including a test to see what effect simulated insect predation would have on growth patterns, and if these patterns could be passed along through generations. A separate experiment was set up to look at the effect that nutrient levels in the parental generation have on the growth of their offspring. In the predation experiment high mortality was found, including the complete decimation of one test population, which forced me to remove this experiment from my research, since I would not have had time
to replant these populations and have enough time to complete this experiment. During this time I also realized that focusing my studies simply on flowering time in relation to light levels would be my best use of time and resources.

The light level experiment involved growing 566 plants at both high and low light levels, with seeds of both different parental conditions (high or low light) being grown in each tray to completely eliminate the effect that incubator positioning may have on the growth of these plants. This portion of the experimentation involved planting two seeds into one cell in order to compare growth patterns and flowering time between plants of two different parents under identical conditions. The planting was done by covering one side of each cell with a ruler while planting one seed and then covering the other half when planting the seed from the other parent. This insured that post planting I could differentiate between which parent each seedling was from. During their growth I recorded the date of bolting as well as the number of leaves on the plant on the date of bolting to determine how sunlight affects this rate, and more importantly if the amount of sunlight received by the parents has any impact on the date of bolting or number of leaves present at time of bolting in their offspring. Once the plants were grown for four weeks following the first bolting in that treatment, two-thirds of the plants were removed and analyzed. Measurements were taken on both the number of seed pods produced, as well as the number of leaves at time of harvest. The other one-third of the plants were left growing to achieve mature seed production in order to allow experiment continuation for future generations.

This experiment was performed both with wild type and drm1, drm2, cmt3 mutant plants, and was designed to provide us with interesting data into whether the amount of sunlight that a plant lives in is somehow epigenetically encoded in a way that will increase fitness in their offspring. For example if we find that plants from parents in 13h conditions are routinely bolting earlier than their 18h nearly genetically identical relatives in the wild type, but not in the mutants, then non-CG methylation, may account for the difference. There are countless other possible scenarios which may transpire in the offspring, and after our data are collected a statistical analysis of flowering time, seed pods produced, plant final height, and leaf number will be completed to see if there is any difference in life history traits on these plants dependent on their parents conditions. The plants in the 13h conditions will be analyzed to see whether plants from high light or low light parents respond differently to this decreased light cycle.

**Solidago rugosa:**

Plants were measured in the field at two distinct populations, one growing in field and adjacent forest on Appalachian Trail land in Hunters Run, PA (on South Mountain eight miles south of Carlisle), and one growing in similar field and adjacent forest at the Reineman Wildlife Sanctuary (on Blue Mountain eight miles northwest of Carlisle). We selected plants randomly by walking until we encountered a *Solidago rugosa* plant, then stretching out a meter stick from that plant in a blindly chosen direction and selecting the *Solidago rugosa* plant nearest to the other end of the meter stick. We used this method because the plants are patchily distributed so one needed to locate a patch and then select a plant without bias within that patch. After selecting plants measurements were made of the total height, height at first branching, number of flower clusters, and stem width.
Seeds were then collected in late November, 2010 from each of 60 plants (30 field (F) and 30 woods (W)) at each of the two sites. The seeds were stored dry in paper packets in a refrigerator until early January, when we identified ten healthy, fertile and undamaged seeds from each parent. Each set of ten seeds was planted on two pieces of wet filter paper in a 90 cm plastic petri plate. The plates were refrigerated or “cold-stratified” for five weeks to bring them out of dormancy and then placed in a growth chamber on a 13:11 hour light cycle at temperatures of 20°C daytime and 15°C nighttime on February 11, 2011. By March 3, most seeds had germinated, and we planted the seedlings in 6-inch plastic pots using Hampden Farms Potting Mix. Four seedlings – one from Hunters Run field, one from Hunters Run woods, one from Reineman field, and one from Reineman woods – were planted in each pot as an experimental unit. In total, 60 pots were planted, using 240 seedlings (four from each of the 60 parents). The pots were placed in the greenhouse. On March 23, half of the pots were placed behind 80% shadecloth (Gempler’s), simulating typical forest shade based on light measurements taken in local forest and field over previous summers. The remaining pots continued to receive full sunlight. The seedlings of each parent were represented equally between the lighted and shaded treatments. On May 9 data were collected from the seedlings, 67 days after they had been transplanted to soil.

Meanwhile, in early December, 2010 60 plants were dug up at Reineman Sanctuary (again, 30 from field and 30 from woods although they were not necessarily the same individuals as those from which seeds were collected.) The plants were each placed in an 8-inch plastic pot using Hampden Farm Potting Soil around the soil-root ball as needed to fill up the pot. The plants were then overwintered in a field several miles north of Carlisle under and within a thick layer of pine bark mulch, to keep the temperature relatively stable. On February 11, 2011, the plants were moved into the greenhouse. Half of each of the F and W plants were placed behind an 80% cover shadecloth, while the other half was fully exposed to the sun in our greenhouse. Data was collected from these plants in late April, approximately 75 days after they had been transplanted to the greenhouse.

**EXPERIMENTAL PROCEDURES**

During the plants’ growth we left all of the seedlings and repotted plants open to the sun for the first week of their growth to simulate canopy openings during the beginning of the growing season. During this time there was also some weeding done on the replanted *Solidago rugosa*. While this artificial selection on competing plants may not occur in the wild, we felt it was necessary in order to make accurate comparisons on the growth patterns of the various plants. Competition for water, nutrients, and light space could all potentially hinder the growth of some plants, and in turn lead to lower growth levels. For the repotted *Solidago* I took measurements of plant height, stem width, and number of central stems produced. These data will be compared with the data collected on these plants in the field. It will be interesting to note if population trends observed in the field will hold when they are potted and placed in a common environment (hinting at genetic population differences), or if we will find that the plants revert to similar growth patterns when brought into a common environment.

The seedling portion of this experiment was done in order to provide us with a data set to compare the potential differences in the levels of epigenetic resetting in sexual versus asexual
reproduction. When comparing the growth of our seedlings I was limited in time by the types of measurements that I was able to conduct due to their small size, and inability to reach maturity within one year. That being said, in the case that we still see differences in the asexually propagated population it will be interesting to see if there are any differences in the growth of the seedlings. If not, it is possible that the methylation was reset in these seeds during meiosis. Data was collected as to the number of plants bolted under the various conditions. For the seedlings that do bolt before the end of the semester I measured the height and width of the central stem in order to develop another set of height: width data points for each of the populations. Comparison of the seedling growth between plants from different descent will provide us with data on the early stages of a plant’s life cycle, which are often the most important in determining whether or not a plant will become established in a given environment.

RESULTS

Arabidopsis thaliana:

Arabidopsis thaliana proved to show phenotypic plasticity in response to varying light for both the wild type plants as well as the mutant strains. After analyzing the data we determined that both number of days to bolting and total number of seed pods produced seemed to show significant variation between the populations in 18 hour light and 13 hour light. The fact that these traits were affected by varying light levels lead us to determine that they would be the most likely traits to be transgenerationally inheritable. These traits are both also very important to the plants fitness, and would therefore be most likely selected on through natural selection.

When analyzing the data between our four populations of wild type plants we used a two way-ANOVA analysis to look for significant differences between the current light cycle the plants were in as well as any effect that parents’ light cycle may have on either days to bolting or seed pods produced. We found that the current light cycle had a significant effect on the number of seed pods produced (F=85.82, df=1, 88 p<0.001), with the plants in 18 (H) of light producing on average about twice as many seed pods as their 13 (H) counterparts (98.45 pods per plant from 18 hour plants vs. 42.41 pods per plant from 13 hour plants). We also found that flowering time was delayed by a significant margin in plants grown in 13 hours of light compared to 18 hours of light (42.3 days 18(H) vs. 48.7 days 13(H)).

These differential growth rates dependent on current light levels are to be expected as they can easily be explained by differential maximum rates of photosynthesis. The impact of parental light levels on current growth was the data I was primarily interested in. Due to high levels of variation in both days to bolting and number of seed pods produced, the parental light affect proved not to be statistically significant, but did seem to show a trend toward higher fitness (increased seed pod production and decreased days to bolting) in plants from 18 (H) light cycle parents (see Table 1 and Figure 1). In terms of seedpod production we found that the parents light levels accounted for some of the variation within the population (F=2.89, df=1,88 p=0.0928). The average number of pods produced by plants from 18(H) parents was over 10% higher than the average number of pods produced from plants of 13(H) parents. The plants from
13(H) parents were also shown to take on average 1.02 days longer to bolt than plants from 18(H) parents (see Figure 2). These differences were also found to not be statistically significant ($p > 0.05$).

The drm1, drm2, cmt 3 triple mutants experienced trouble during the growth process due to rapid drying out, decreased flowering rates, and lower germination rates. They were eliminated from the experimental portion of our study, but maintained in order to isolate seeds for future experiments.

**Solidago rugosa**: Field Results:

The data collected from our natural field populations showed variable growth patterns between both plants growing under canopy coverage versus those found in a field setting, as well as a significant difference in growth patterns between our two sites. Data were analyzed using a two way-ANOVA to look at variation in quantitative measurements with multiple variables in one test. Data analysis showed that height, width, and side branches produced were the most distinct characteristics between the various populations. In order to best analyze the height and width of the plants we decided to use height:width ratio. This statistic will show us how the plant allocates its resources between vertical growth and stability-enhancing stem horizontal growth. When looking at number of branches between populations we used the ratio of branches:volume to take into account the differences in overall plant vigor when analyzing how many branches each plant would produce (see Table 2).

The data here showed conclusively that the Height:Width ratio of the Rough-Stemmed Goldenrods was much higher in woods sites compared to field sites ($F=142.68$, df=1, 116 $p<0.0001$), as well as significantly higher at Hunter’s Run compared to Reineman Sanctuary ($F=15.12$, df=1, 116 $p=0.0002$). The average values for height to width data between our four sites can be seen in Figure 3, and the height and width of each individual plant sampled is shown in Figure 4.

The volume data used for the branch:volume analysis was an estimate of the central stem volume calculated by using the formula for volume of a cylinder. Plant stem width was used to determine the radius, and the central stem’s total height was used as the height value. The two way-ANOVA was done using branches +1 in order to transform our data so that plants with zero branches did not negate the effect of plant height from the equation. It was found that more branches were produced per volume ($cm^3$) in plants found in a field environment than those from a wooded environment ($F=5.49$, df=1, 116 $p=0.0208$), and that plants from Reineman Sanctuary had significantly more branches per volume than did plants from Hunter’s Run ($F=10.04$, df=1, 116 $p=0.0020$). The average value for branches:volume can be seen in Figure 5 and the individual data points can be seen in Figure 6.

It is also important to note that for both sets of these measurements there was not shown to be any difference in interaction between Habitat and Site between these data points. For example the effect that the different habitats had on height:width at Reineman was no different than the effect that the different habitats had on height:width ratio at Hunter’s Run.
Solidago rugosa: Greenhouse Results: Dormant Transplants

Because the plants were not able to be grown to a point where a sufficient number of the individuals had developed side branches we used height and width data as our sole data points. Once again we transformed these data into a height: width ratio. The four populations analyzed for variable growth patterns were as followed: plants that were dug up from the woods and replanted in full sun, plants that were dug up from the woods and replanted behind 80% shade cloth, plants that were dug up from the field and replanted in full sun, and plants that were dug up from the field and replanted behind 80% shade cloth.

We found that the habitat from which the plants were dug up had no effect on the next season growth of the plant (F=0.01, df=1, 116 p=0.9163), but that the current light conditions did significantly impact the height: width ratio of the plant (F=44.03, df=1, 116 p<0.0001). In Table 3 and Figure 7 we see that the height: width ratio is higher for plants behind shade cloth than for those plants exposed to full sunlight. This suggests that the shade avoidance response can be elicited through decreased light levels without any change to the type of light that has reached the given plant.

Solidago rugosa: Greenhouse Results: Seedling Growth

The extent to which we could gather statistically significant data on relevant traits was limited by the fact that few plants bolted that were either behind the shade cloth, or of Reineman descent. In total, of the 92 seeds planted from Reineman, only 5 of the seedlings had bolted within 65 days of planting compared with 27 of 92 seedlings from Hunter’s Run (see Figure 8). A Fischer’s Exact Test showed that seeds collected at Hunter’s Run were significantly more likely to bolt within 65 days than were seeds harvested at Reineman (p>0.001 df=238). These data did provide us with some unexpected and interesting data that will be analyzed in the Discussion section, but the shortage of bolting plants prevented us from making any meaningful comparisons between the growths of the central stems between these sites. Interestingly when analyzing the height: width ratio of plants grown from seed we saw no significant difference in ratio between the plants grown behind shade cloth and those exposed to full sunlight (F=0.04, df=238 p=0.8369). It was also found that once again the parents-light conditions did not play a significant impact on the offspring’s height: width ratio (F=0.96, df= 238 p=0.3367). This can be seen in both Table 4 and Figure 9.

DISCUSSION

Arabidopsis thaliana:

The growth patterns of plants in an 18 hour light cycle versus those in a 13 hour light cycle showed to be significantly different. The level of phenotypic plasticity in response to the
changes in light cycle proved to be difficult to determine from the data collected in this portion of our experiment. This was due to it being a challenge to compare two populations receiving different amounts of light energy. We found that the plants took longer to reach maturity under the decreased light cycle, and that fewer seedpods were produced. The lowered number of seedpods, yet unchanged number of leaves, shows similar levels of vegetative growth with reproductive growth being sacrificed. As phenotypic plasticity can be defined as "environmentally dependent phenotypic variation in the growth or development of an organism," we can treat this variable allocation of resources as phenotypic plasticity (Holeski, 2007). This decrease in number of seed pods produced, with the maintenance of similar levels of vegetative growth has been shown previously, and in at least one other case it was found that there is an increase in the overall leaf mass from plants in low light (Pigliucci and Kolodynski, 2002). That experiment was done with decreased light levels on the same light cycles. *Arabidopsis thaliana* has been described as a plant that is not particularly plastic, probably due to it's incredibly rapid reproductive cycle, allowing for little change in its highly successful growth patterns. It is important to note that this low phenotypic plasticity does not mean the plant is not variable within species. In fact it has been found that this species is highly variable genetically, and these different genetic populations do in fact have many different growth patterns (Pigliucci and Kolodynski, 2002).

Decreased allocation of resources toward reproductive structures in low light can be an evolved mechanism in that a given plant must first become vigorous enough at a vegetative level before it will be able to support reproductive structures and successfully flower. Another potential explanation of this phenomenon is that the plants are building up a large collection of basal leaves so that when an opening in the canopy does appear they will be able to bolt, flower, and set seed with great efficiency. The increased number of seed pods produced compared to overall growth in high light plants could also be an example of natural selection at a group level. For *Arabidopsis thaliana* in its natural environment the plants receiving more light would be the plants that are more successful, in that they either germinated faster, or happened to land in a more advantageous position. Either way this plant can be seen as more "successful" (I will define success as the overall growth of the plant, and potential "fitness" of the individual, not the actual number of offspring produced. This differs from the typical definition of fitness which assumes that an organism produces a maximal number of offspring) than a competing plant receiving lower light (note that this increased success can simply be due to the fortune in landing in a canopy opening, and not any specific genetic superiority). The plant receiving more light, the more successful plant, has been shown in laboratory experiments to produce more seeds per overall plant growth than less successful plants, those receiving less light (Pigliucci and Kolodynski, 2002). It has also been shown that number of leaves produced (vegetative growth) usually has an inverse relationship with flowering time, meaning that plants with more leaves tend to take longer to bolt. This could be due to group level natural selection favoring those populations in which plants receiving more light produce disproportionally (in comparison to overall successfullness as an individual) more seeds then those plants receiving less light. These populations may become a more "successful" population faster than a population in which both low light and high light plants produce comparable numbers of seeds. This disproportionate reproduction and addition to the next generation's gene pool by the most successful plants will
Plasticity and Epigenetic Inheritance in Response to Light Levels

concentrate the best genes and gene/environmental niche combinations more rapidly than if any individual capable of reproduction was allowed to reproduce at their maximal rates.

This theory would fall under the category of "group selection", and would come under immediate scrutiny as this ability for a plant to act altruistically with its neighbors has never been proven. The neighborhood model of group selection would be the most likely form in this case as it explains the development of altruistic characteristics by the likelihood that organisms nearby are closely related (Wilson, 1987). Note that this example of group selection would be in the opposite direction of how group selection is usually discussed in that rather than a plant with great vigor altruistically allowing nearby plants to be more successful, we instead would see less successful plants act altruistically toward the population in that they are not diluting the gene pool with their less favorable genes (their genes are less favorable in the current environment). The most likely organisms to express this form of group selection would be plants (since they cannot move and are therefore often closely related to their neighbors), with high genetic similarity within a given population, and high population densities in which there would not be enough resources for each future seed to develop. This potential system of group selection would theoretically provide an increase in fitness in the following situation:

1. Very large population of plants, in a variable environment.
2. Across variable environment we see some plants show greater success than nearby plants.
3. Seeds from the more successful parent plants are more likely to be successful than seeds from less successful parent plants.
4. There are not enough resources so that each seed can grow to maturity.
5. If a disproportional (in comparison to overall successfulness of the individual) percentages of seeds come from the most successful plants in the population then the population will be comprised of a higher percentage of plants from successful parents.
6. This population will achieve greater fitness at a faster rate than a population in which all plants contributed similar number of seeds to the future generation.

In order to test the potential validity of this theory we would need to show that Arabidopsis thaliana plants in low light levels can be made to, and have the ability to, produce seeds at higher levels. It could also be shown that group selection would act directly on slowing down the time of bolting and flowering, first allowing the more fit plants to set seed before those receiving less light do. If it can be shown that there is a control mechanism (potentially through methylation) to decrease seed production, or delay bolting in low light situations, it would be a sign that group selection by decreasing the input of less fit individuals to future populations may be occurring. This is assuming that faster bolting and increased seed pod production are in fact two traits that will increase the amount of genetic contribution to the next generation that the given individual will have. An experiment looking at genes such as EMF1 and EMF2 that are required to maintain vegetative growth and prevent early flowering would be interesting genes to study. It is important to note that this system may only be beneficial for a very small portion of the plant world, and findings that this form of group selection is occurring in a given species would not mean that it is a widespread evolutionary mechanism. Along with epigenetics, the
maternal effect could allow for mother plants receiving more nutrients, to put more energy into the seeds of their offspring (Roach and Wulf, 1987). Since increased nutrient levels does not actively change the transcription levels of genes it cannot be placed into the same category as methylation or histone modification. Still, it would allow for more successful maternal plants to pass on a competitive advantage to their offspring, and therefore fit into the category of group selection. Lastly, the delayed flowering could also be beneficial for the overall population because it would spread out the time in which seeds were dispersed, preventing a temporary drought or something of that nature from wiping out all of the seeds for next generation. Also, it is important to note that within the given populations we would have to see high levels of genetic similarity between individuals in order for this theory to cooperate with the selfish gene theory (Dawkins, 1976).

The effect that parent light levels have on offspring growth patterns or fitness was not shown to be quite significant in our results, but did seem to show a trend toward plants from high-light parents producing more seeds than identical plants from low light parents. High variability and lower than expected survival and bolting rates lead to our results being inconclusive, but further studies could be very interesting and important if they do in fact show this increased fitness can be passed along through generations. Further studies will have to be performed using identical test procedures, and introducing more wild type ecotypes than just the Columbia strain used in this experiment would be beneficial. This experiment could also be potentially altered in numerous ways. For example if the plants grown in high light could be tagged with a genetic marker that would be passed on to their offspring, we could take those seeds and the low light seeds, grow them in a more traditional competitive experiment, and be able to test which plants survived by the marker in the high light line of the plants. While it has been shown that plants do show a certain level of phenotypic plasticity to light, it has not been established conclusively that these traits can be passed on to their offspring, so until those results are either discredited or further proven it is unwise to postulate on the potential implications of this finding. One interesting thing to look for though is a change in methylation states in higher light that may code for those plants to bolt earlier or simply produce more seeds. If this is the case, we may see those methylation patterns inherited by the offspring, leading to the offspring of high light plants also bolting faster or producing more seed pods. In this case we could say there is “inheritable success” in a sense in that seeds from a successful parent could have an edge on reproductive fitness from a seed from a less fit parent, even if their genomes are identical. This could go along with my earlier conceptual theory on group selection if we can show that even given similar success in the current generation, plants whose parents were more successful are more likely to produce more successful offspring than plants with less successful parents. A population of the type I described in my earlier example would then be more successful if it did develop a system of “inheritable success.”

**Solidago rugosa:**

Our results from both field and greenhouse analysis on the growth patterns of *Solidago rugosa* showed very plastic growth patterns in response to shade. The growth patterns in shaded conditions were consistent with the “shade avoidance response” where we see that plants in a
Plasticity and Epigenetic Inheritance in Response to Light Levels

shaded environment tend to grow taller, thinner, and develop fewer side branches. We found that all three of these growth patterns were found in plants not only growing under canopy cover, but also those in a controlled environment in which plants were placed behind shade cloth. While shade cloth has been shown to induce the shade avoidance response in certain plants, such as tomatoes (Hattrup et al., 2007), most ecological and mechanism based studies have stressed the importance of far red: red wavelength light reaching the plant being key for inducing this response (Franklin, 2008). Far red: red light levels vary as canopy cover begins to increase due to the fact that far red is not absorbed by plant leaves, while red light is. This ability to sense changing far red: red light would give plants an early way to detect oncoming shade (Franklin, 2008). In our study in the greenhouse we see that an overall decrease in light levels can also cause a similar shade avoidance response. This may be due to interactions with cryptochromes which respond to overall levels of Blue wavelength light, compared to the better studied phytochromes which seem to elicit downstream responses based on variable ratios of light of different wavelength (Franklin, 2008).

It is also interesting to note that we saw very distinct growth patterns between plants from Reineman Sanctuary and Hunter's Run. We did however, find that both sites showed similar shade avoidance responses, showing similar plasticity, but their original growth patterns demonstrated that these two populations are in fact distinct. In other words, the interaction between site and habitat was not significantly different between our populations. When looking at Height/Width ratios, for example, we found that the effect the habitat had on growth patterns was the same for both sites ($F=1.28$, $p=0.261$). This variable plasticity between populations is important as higher levels of phenotypic plasticity can give one population "enhanced ecological niche breadth because plastic responses allow advantageous phenotypes in a broader range of environments" (Richards et al., 2006). It would be very difficult to argue for any purely genetic cause for the variable growth patterns between sites if we had not brought seeds from both sites back to the greenhouse. This is due to the potential that the differential growth patterns between locations could be explained as a plastic response to different conditions (such as moisture, or nutrient levels) that were not under our control.

The seeds brought back into the greenhouse gave us some very intriguing data into some potential genetic differences in these two populations. While the fact that almost none of the Reineman seedlings bolted after 67 days prevented us from analyzing the height to width ratio between the two populations, it did lead to us making the observation that seedlings from Reineman Sanctuary: either bolted later, or simply at a lower rate, than do plants from Hunter's Run. This could show that either that there is a genetic difference that leads to the seedlings from Hunter's Run bolting earlier than the seedlings from Reineman, or there is a genetic difference that leads to plants from Hunter's Run putting more energy into their seeds, allowing those seeds to bolt earlier. By analyzing the seed weight and composition we could determine if more energy is in fact put into the seeds from Hunter's Run parents, or whether the earlier bolting is simply due to a difference in bolting leading to different types of seedling growth. It would also be necessary to measure seed weights from plants of both populations grown in controlled environments in order to ensure the variable allocation of resources to seed production is of genetic consequence.

It is hard to hypothesize the potential evolutionary mechanisms for driving one population to produce seeds that bolt faster than seeds from a different site if we are unable to answer the previous question of what is causing one population of seeds to be delayed in bolting.
If we do find that increased energy is put into sexual reproduction in the Hunter's Run populations, it may be that this population is more dependent on sexual reproduction than the population from Reineman Sanctuary. Another possibility could be that the Hunter's Run population is younger, meaning that it got colonized (through sexual reproduction) more recently. This would mean that the population would be more closely related to the original colonizers which would have been selected for their ability to colonize, from seed, more successfully, rather than through asexual reproduction, as they reproduce in established populations.

Interestingly, we see no significant difference in height to width ratio when comparing seedlings grown behind shade cloth and those not behind shade cloth. This is likely due to the shade avoidance response not setting in until slightly later in the plants' growth cycle. If we do find that plants grown from seeds behind shade cloth show no shade avoidance it would be quite interesting. This would be unexpected, as the plants dug up from both the woods and the field did show this response when grown behind shade in the greenhouse.

In this portion of the experiment we were able to quantify the shade avoidance response in Solidago rugosa as a clear example of a plastic response to variable light levels in plants, discover that a change in wavelength ratios is not necessary to induce this plastic response, and collect field phenotypic data as well as greenhouse seedling data suggesting that these two populations of Goldenrods are quite genetically distinct from one another. In this plant we have not however, found any sign of transgenerationally environmentally induced epigenetic changes on the phenotypic level.

ACKNOWLEDGMENTS:

Many thanks to my committee of Professors Carol Loeffler, Thomas Arnold, and Kirsten Gus for continued support throughout my research. Original inspiration into studying epigenetics is given to a great lecture by Professor Michael Roberts. Finally, special thanks to my advisor Carol Loeffler for guidance, faith, support, and helpful questioning of my project design and theoretical intuition from day one until completion.
TABLES AND FIGURES

Table 1. Average values for three traits in wild-type (COL) Arabidopsis thaliana at various light conditions and from different parent conditions

<table>
<thead>
<tr>
<th>Light (Parent’s Light)</th>
<th>Number of Seed Pods</th>
<th>Days to Bolt</th>
<th>Leaves at Bolting</th>
</tr>
</thead>
<tbody>
<tr>
<td>18H (18H)</td>
<td>105.56</td>
<td>41.60</td>
<td>14.23</td>
</tr>
<tr>
<td>18H (13H)</td>
<td>91.54</td>
<td>43.06</td>
<td>14.00</td>
</tr>
<tr>
<td>13H (18H)</td>
<td>45.72</td>
<td>48.38</td>
<td>15.03</td>
</tr>
<tr>
<td>13H (13H)</td>
<td>39.16</td>
<td>49.16</td>
<td>15.52</td>
</tr>
</tbody>
</table>

Table 2. Average values of Solidago rugosa phenotypic data collected at various field sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Stem Diameter (mm)</th>
<th>Height at First Branching (cm)</th>
<th>Total Height (cm)</th>
<th>Number of Branches</th>
<th>Height/Width</th>
<th>Branches/Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reinerman</td>
<td>3.66</td>
<td>61.15</td>
<td>89.24</td>
<td>5.43</td>
<td>24.38</td>
<td>0.144</td>
</tr>
<tr>
<td>Hunters Run</td>
<td>3.63</td>
<td>78.79</td>
<td>103.10</td>
<td>3.15</td>
<td>28.40</td>
<td>0.0742</td>
</tr>
<tr>
<td>Habitat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woods</td>
<td>3.38</td>
<td>85.68</td>
<td>106.93</td>
<td>2.70</td>
<td>31.68</td>
<td>0.0693</td>
</tr>
<tr>
<td>Field</td>
<td>3.92</td>
<td>56.81</td>
<td>85.41</td>
<td>5.88</td>
<td>21.82</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Table 3. Average values for Solidago rugosa dug up at various sites and replanted in the greenhouse. Phenotypic data were collected from plants grown in greenhouse in either full sun or behind 80% shade cloth.

<table>
<thead>
<tr>
<th>Light (Parents Habitat)</th>
<th>Number of Plants Sprouted</th>
<th>Height (cm)</th>
<th>Width (mm)</th>
<th>Height/Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Sun (Field)</td>
<td>25</td>
<td>18.26</td>
<td>2.32</td>
<td>7.87</td>
</tr>
<tr>
<td>Full Sun (Woods)</td>
<td>32</td>
<td>18.48</td>
<td>2.38</td>
<td>7.75</td>
</tr>
<tr>
<td>Shade (Fields)</td>
<td>25</td>
<td>24.43</td>
<td>2.14</td>
<td>11.40</td>
</tr>
<tr>
<td>Shade (Woods)</td>
<td>24</td>
<td>25.7</td>
<td>2.30</td>
<td>11.15</td>
</tr>
</tbody>
</table>
Table 4. Average values of Solidago rugosa grown from seeds of plants found at various sites and replanted in the greenhouse. Phenotypic data was collected from plants grown in greenhouse in either full sun or behind 80% shade cloth.

<table>
<thead>
<tr>
<th>Light (Parent’s Habitat)</th>
<th>Number of Plants Bolted</th>
<th>Height (cm)</th>
<th>Width (mm)</th>
<th>Height/ Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Sun (Field)</td>
<td>11</td>
<td>11.77</td>
<td>2.18</td>
<td>5.40</td>
</tr>
<tr>
<td>Full Sun (Woods)</td>
<td>11</td>
<td>11.53</td>
<td>2.15</td>
<td>5.35</td>
</tr>
<tr>
<td>Shade (Field)</td>
<td>4</td>
<td>7.18</td>
<td>1.58</td>
<td>4.56</td>
</tr>
<tr>
<td>Shade (Woods)</td>
<td>6</td>
<td>8.35</td>
<td>1.38</td>
<td>5.96</td>
</tr>
</tbody>
</table>

Figure 1. Average number of seed pods produced by Arabidopsis thaliana plants grown in different experimental conditions and from different descent. Plants were allowed to grow for four weeks after the first plant of that type had bolted before seed pod counts were taken. Data were collected from 18H plants from 18H hour parents (n=27), 18H plants from 13H parents (n=28), 13H plants from 18H parents (n=18), and 13H plants from 13H parents (n=16). Variable sample sizes were due to variable germination rate, bolting rate, and plant death during growth. The current light level was found to have an extremely significant effect on the number of pods produced (F=85.82, p=<0.001), and the effect of parent light levels had a not quite statistically significant impact on the number of seed pods produced (F=2.89, p=0.0928).
Figure 2. Number of days to bolt for *Arabidopsis thaliana* plants grown in different experimental conditions and from different descent. Plants were checked daily to determine the precise day on which they had sent up a central stem (bolted). After three weeks of growth following the first bolting of a plant in the given treatment I abstained from collecting data on days to bolt because by this time the number of plants bolting had decreased rapidly, and it was determined that any plants to bolt at this point would have been sufficiently delayed compared to its competitors to successfully flower in the wild. Data were collected from 18H plants from 18H hour parents (n=46), 18H plants from 13H parents (n=46), 13H plants from 18H parents (n=26), and 13H plants from 13H parents (n=24). It was found that there was a significant increase in days to bolting in plants in 13H light vs. those in 18H light. We did not however find that there was a statistically significant effect on flowering time based on the parent’s light condition.
Figure 3. Average Height/Width ratio for *Solidago rugosa* plants at two separate sites and at two distinct habitats at each site.

For each of the four sample sites data were collected randomly from thirty plants at each habitat at each of the two sites (n=30). There was found to be a statistically significant difference in Height/ Width ratio based both on the habitat that the plants were taken from (F=142.68, p=<0.0001), and the site from which they were collected (F=15.12, p=0.0002). Note that a higher Height/ Width ratio corresponds to taller, thinner plants.

- Reineman Woods = 29.5 cm^2/mm
- Reineman Field = 20.5 cm^2/mm
- Hunter's Run Woods = 33.6 cm^2/mm
- Hunter’s Run Field = 23.3 cm^2/mm
Figure 4. Coordinate plot of *Solidago rugosa* plants found at Hunter’s Run and Reineman comparing stem width (mm) vs. height of central stem (cm). Individual plants from each of the four populations are shown plotting both the plant’s height in centimeters, and stem width in millimeters. Plants from Hunter’s Run were typically taller and thinner than plants from Reineman, and plants from a wooded habitat were taller and thinner than plants from the field.
Figure 5. Average Branches/Volume ratio for *Solidago rugosa* plants at two separate sites and at two distinct habitats at each site.

For each of the four sample sites data was collected randomly from thirty plants at each habitat at each of the two sites (n=30). Branches measurements were transformed into branches + 1 in order to prevent plants with 0 branches from having their branches/ volume value removed from the equation. It was found that more branches were produced per volume (cm$^3$) in plants found in a field environment than those from a wooded environment ($F=5.49$, $p=0.0208$), and that plants from Reineman Sanctuary had significantly more branches per volume than did plants from Hunter’s Run ($F=10.04$, $p=0.0020$). These data, in conjunction with the previous data on plant height/width ratios, show that plants that tend to be shorter and wider also tend to be those that have more side branches/ volume.

Reineman Woods = 0.1432 Branches +1/ Volume (cm$^3$)
Reineman Field = 0.1886 Branches +1/ Volume (cm$^3$)
Hunter’s Run Woods = 0.0652 Branches +1/ Volume (cm$^3$)
Hunter’s Run Field = 0.1402 Branches +1/ Volume (cm$^3$)
Figure 6. Coordinate plot of *Solidago rugosa* plants found at Hunter's Run and Reineman comparing volume (cm$^3$) vs. number of branches. Individual plants from each of four sites is shown plotting the volume of the plants vs. the total number of side branches produced. The trend lines shows that as the volume of a given plant increases in a field setting, it develops side branches more rapidly than a plant in the wood setting. We also see that while the slopes between Hunter's Run and Reineman are similar in either habitat, Reineman plants do, on average, have more side branches than do plants from Hunters Run.
Figure 7. Average Height/Width ratio of *Solidago rugosa* plants dug up from both field and wood sites at Reineman sanctuary and replanted in either full sun or behind shade cloth. Approximately 75 days after our plants were moved into the greenhouse height and width measurements were taken on each of the plants that had bolted. In total 25 plants had risen from both the field transplants in full sun, and field transplants behind 80% shade cloth, while 32 plants had risen from woods transplants in full sun and 24 from woods transplants behind 80% shade cloth. The effect that current light levels have on height/width ratio was found to be statistically significant (F=44.03, p=<0.0001), and consistent with our findings in the field that plants under canopy cover will grow taller and skinnier than those in an open field.
Figure 8. Number of seedlings from Hunter's Run and Reineman descent that had or had not bolted after 67 days of growth.

This figure clearly displays that many more plants of Hunter's Run descent had bolted by the date of the data collection than plants of Reineman descent. The effect of descent was shown to be extremely statistically significant using a Fischer's Exact Test ($p > 0.001$).
Figure 9. Height/Width ratio of seedlings grown either in full light or behind 80% shade cloth, and from either parents in a wooded or field environment.

The data collected from our seedlings after 67 days of growth showed no significant patterns in variable height/ width ratios between populations. The low bolting counts - woods parents in full light (n=11), field parents in full light (n=11), woods parent in 80% shade (n=6), and field parent in 80% shade (n=4) - could be why no trend was observed.