Phenotypic Plasticity as a Slow Evolutionary Response to Directional Climate Change

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ABSTRACT

The plants grown in the sun and shade do exhibit qualitative traits of phenotypic plasticity, however, careful analysis of quantitative traits show that there is little to no difference. The direction of plasticity that was expected was more towards an extreme end in which the organism, in this case, the two plants would specialize distinctly in the two given environments. The objective of this study was to determine whether phenotypic plasticity exists between the same plant under different environmental conditions. The results show that there are specific features of a plant, like the weight of the bore, that show phenotypic selection. Additionally, there was no adaptive change found between the number of stoma average/$100um^2$ of *E. japonicus* and *V. rossicum* under the sun and shade conditions. The significance of the finding is that there are no big differences between the plants observed that show that phenotypic plasticity is a big response to directional climate change overtime.

INTRODUCTION

As environments change, an organism must respond and specialize to their unique environments. This is when phenotypic plasticity comes in, it includes morphological and physiological changes in an organism's behaviour due to environmental changes. With climate change occurring, phenotypic plasticity is a mechanism that an organism uses to respond to the rapidly changing environment (Kingsolver et. al., 2017). In order to understand the general field of study denoting phenotypic plasticity and how organisms adapt to changes in their environment, it's important to narrow down and focus on a specific environmental condition that an organism may face. An example of such an environmental condition is the absence or presence of sunlight in plants. Under low-light conditions such as one that a shade-leaf may face, photosynthesis is limited by light availability. A sun-leaf would be limited in conserving its water-uptake. By looking at sun- versus shade-leaves we would be able to understand whether phenotypic plasticity acts as an adaptive or non-adaptive evolutionary response to directional climate change. I hypothesize that the existence and nature of plasticity in plants is adaptive to its environment and that it's advantageous for plants experimentally grown under sunlight and those grown in the wild under the sunlight to maintain the observed phenotypic characteristics that would help them in their environments. Additionally, it would be advantageous for the plants experimentally grown under the shade and the wild-type grown under the shade to have similarly observed phenotypic characteristics. Moreover, I hypothesize that the observed adaptive changes between the sun- and shade-leaf are evolutionary responses to directional climate change because those favourable traits would be selected for under those specific environmental conditions.

Recent studies have shown that plasticity depends on the magnitude of seasonal variation in climate relative to interannual variation (Kingsolcer, 2017). Other studies have shown how phenotypic plasticity is expressed at the level of modular subunits, individual meristem

responses of plant responses that are triggered by environmental conditions (Hans de Kroon et. al., 2005). Moreover, these local responses by the plant rules in favour of environmental variation and so the modular interaction rules are evolving traits targeted by natural selection (Hans de Kroon et. al., 2005). Furthermore, this would imply that it's not the whole plant that's responding to environmental change, but rather parts of the plant interacting with each other and the changing environment.Other studies have shown that changes in temperatures affected by abiotic conditions or even maternal environmental conditions, show responses in the next generation (Weinig, 2000). The purpose of this experiment is to look at phenotypic plasticity by looking at lab-grown cloned plants (*Euonymus japonicus)* and the natural wild-type Dog-Strangling Vine (*Vincetoxicum rossicum)* as found in nature. Both grown under the absence and presence of sun, with the phenotypic plasticity being represented by the difference in observed characteristics between the plants under the different light treatments.

MATERIALS AND METHOD

The two species that were examined under the two conditions included; *E. japonicus* x sun, *E. japonicus* x shade, *V. rossicum* x sun and *V. rossicum* x shade. In this experiment there were two methods of data collection; qualitative and quantitative. Qualitative differences included noting down obvious physical differences that are observed between the leaves grown under different light regimes. The qualitative differences observed are as follows; for *E. japonicus* x sun; small leaves were observed, light green with yellow area, waxy and thick with short petioles, leaves were angled outwards with 45-30. For *E. japonicus* x shade; larger leaves with more green coverage, no waxy appearance, thinner leaves angled straight outwards at 90 degrees and longer petioles. *V. rossicum* x sun have lots of dark-green- colored leaves, small and angled slightly upwards less than 90 degrees, short petioles, thicker waxy leaves. Lastly, *V. rossicum* x shade have less seed production, lighter-green colored large leaves angled outwards at 90 degrees, less waxy in appearance and feel thinner

The quantitative differences include the following techniques to determine leave weight (by area), chlorophyll concentration and the number of stomata. To determine the leaf weight per unit area, we placed a leaf on a Styrofoam pad on the ventral side with the bottom side-up. Avoiding all major veins, we cut 4 bores using the 1cm diameter bore. The next step was to measure the weight of all the 4 bores combined, this was done by placing the 4 bores on an analytical scale. The total lead area of the 4 samples was calculated using the area of a circle - $\pi 2$. The 4 bores were saved for the next step, determining chlorophyll context. To measure the chlorophyll content of leaves we extracted pigment molecules such as chlorophyll a and chlorophyll b from leaves using methanol. The first step for methanol extraction of total chlorophyll (a + b) involved grinding up the same 4 bores in 2mL of 90% methanol using a mortar and pestle for approximately 5 minutes. This process required a lot of arm-work, to grind the paste so that no chunks remain. Using a 10CC syringe, the methanol content was measured and added to a glass vial. An additional 10mL of methanol was added to rinse out the mortar so that there is no solution remaining on the mortar. The glass vial was then labelled and placed on ice for 20minutes to allow time for extraction of the pigment. After the 20minute wait, the vial

was removed from the ice and inverted approximately 8-10 times and poured through a filter, a glass funnel with a kimwipe as a filter, into a 25mL graduated cylinder. The exact volume was then measured and recorded. The extract was then poured into a labeled cuvette for chlorophyll absorbance measurements. Using a spectrophotometer, the absorbance of the chlorophyll extract was measured. The spectrophotometer was first set to zero using a blank cuvette containing only 5mL of 90% methanol, with the wavelength set to 652nm. The next step is to measure the chlorophyll absorbance by measuring the extract into a cuvette, it's important to not touch the sides and to only handle the top of the cuvette. After taking down the absorbance, the sample was disposed of in the waste container provided. The next step involved calculating the chlorophyll content by using the following formula: $c = A / (\varepsilon \times l)$ where A= absorbance, ε= the extinction for coefficient for chlorophyll, l= path length, or the distance at which light travels across the cuvette (=1cm). The formula to compute the chlorophyll content (C) is as follows:

$$\text{Chlorophyll content } (\mu g/cm2) = \frac{V\ (mL) \times c\ (\mu g/mL)}{\text{leaf area } (cm2)}$$

Next we determined the number of stomata by making stomatal casts, and counting the number of stoma per unit area with a microscope. The number of stomata observed was observed from 3 different areas per leaf. The protocol for this procedure involved applying clear nail polish to the ventral surface of the leaf, ideally the same leaf that was used for bores. Avoid the veins when applying the clear nail polish and let it dry. Obtain a frosted microscope slide and label it appropriately with a pencil only. Once the nail polish is dry, take a small piece of clear tape and press it gently against the area covered in nail polish. Remove carefully and stick the tape onto the labelled slide. To determine the stomatal density, the stomal counts need to be done at 400x magnification using the 40x objective. When putting the slide under the microscope, ensure that the 4x objective is in its place first and then refocus if necessary. For each cast, find an area where stomata are present at the highest density and count. Count 3 separate areas of stomata for each castan enter the data in the data sheet.

An assumption that I made is that the difference in absorbance and stomata count will vary between the sun and leaf-plant, specifically the shade-leaf will have a lower absorbance because it receives reduced amounts of red light versus the sun-leaf (Weinig, 2000). Additionally, I assumed that the stomata count will be lower for sun-leaf because it would need to retain it's water and would have less stomata to avoid transpiration.

RESULTS

Some of the patterns/trends that we found are as follows; Figure 1. Average bore weight (g) of *E. japonicus* and *V. rossicum* under sun and shade conditions for the 2019 growing season. The average bore weight was highest for *E. japonicus* in the presence of light and lowest for *V. rossicum* in the absence of light. *E. japonicus* in the absence of light was ranked 2nd on average bore weight (g) and *V. rossicum* in the presence of light was 3rd (Figure 1.). We found that the

stoma average/ $100um^2$ of *E. japonicus* and *V. rossicum* under the sun and shade conditions ranged between 25 and 31 (Figure 2). The stoma average/ $100um^2$ for *E. japonicus* in the sun condition was 31. 89 and in the shade condition it averaged 31. 36 (Figure 2.) The stoma average/ $100um^2$ for *V. rossicum* in the shade condition was the highest, 33.21 in comparison to the sun conditioned *E. japonicus* (Figure 2). The lowest counted stoma average/ $100um^2$ was for *V. rossicum* under the shade condition at 25.53 (Figure 2). Moreover, we found that the average absorbance at 652nm was highest for *V. rossicum* under the sun condition at 0.653 (Table 1). The average absorbance at 652nm was lowest for *E. japonica* under the shade condition at 0.397 (Table 1). Keeping in mind that the absorbance is directly proportional to the concentration (c) of the solution of the sample used, the absorbance value lets us know how much incident light is absorbed by the solution. A low absorbance would mean that the solution is less concentrated compared to a high absorbance in which there more molecules for the light to interact with.

DISCUSSION

It was expected that the observed differences between the sun and shade- leaf would constitute adaptive changes that are evolutionary response to directional climate change because those favourable traits would be selected for under those specific environmental conditions. However, compared to the initial hypothesis, the observed adaptive changes between the sun- and shade-leaf are not as distinct as it was expected to be. Under the sun-conditions it was expected that the sun-leaf would be limited in conserving its water uptake and hence have a lower stoma count to avoid transpiration. Figure 2 shows the stoma average/$100um^2$ of *E. japonicus* and *V. rossicum* under the sun and shade conditions. In the sun conditions the stoma average/ $100um^2$ for *E. japonicus* was higher at 31. 89 versus the shade condition that was 31.36. Additionally, Figure 2 also showed that the lowest counted stoma average/ $100um^2$ was for *V. rossicum* under the shade condition at 25.53. This difference does not prove that there is an adaptive change between the sun and shade- leaf plants.

In a study that looked at the correlation between phenotypic plasticity and adaptive evolution as a response by the species to climate combined recent (1960-2010) climate and phenotypic darta with microclimate, heat balance, demographic and evolutionary models for the montane butterfly, *Colias eriphyle* (Kingsolver et. al., 2017). The phenotype observed was the wing solar absorptivity that responds plastically to developmental (pupal) temperatures and plays a central role in thermoregulatory adaptation in adults (Kingsolver et. al., 2017). Moreover, they found that seasonal changes in weather generate variation in phenotypic selection (Kingsolver et. al., 2017). This study showed that the variation in phenotypic selection cannot be determined through one generation and hence phenotypic plasticity must be observed over a span of time. Likewise, it's possible that the experiment conducted didn't show a distinct difference between the same plant under two conditions because there isn't enough data on the plant over a span of time. Maybe if the plant was observed under the two conditions over a span of time, more variation in phenotypic selection would be observed.

Another literature that also suggests that phenotypic plasticity is an adaptive evolutionary response observed variability in stem elongation when far-red wavelengths (R:FR) was introduced (Weinig, 2000). This study found hypocotyl, or the stem of a germinating seed, responsiveness to R:FR under the high temperature treatment (Weinig, 2000). Furthermore, this study showed that the trait of having an elongated hypocotyl was found in cornfield populations under those conditions relative to populations from weedy site types (Weinig, 2000). This study looked at a specific feature of the plant that varied in phenotypic selection. Looking at the average bore weight (g) of the two plants, *E. japonicus* and *V. rossicum* under sun and shade conditions for the 2019 growing season. The average bore weight was highest for *E. japonicus* in the presence of light and lowest for *V. rossicum* in the absence of light (Figure 1). This suggests that there are specific features of a plant,the weight of the bore, that is favoured under a specific condition.

A possible source of error experimental design that can be done include errors in handling the cuvette during the process of determining the absorbance. This could include accidentally touching the sides. Additional research that I want to conduct to extend this study would be to observe the *V. rossicum* under the same environmental conditions overtime to see if there are any phenotypic changes due to any changes in the environment. In addition to this, I want to see if *E. japonicus* would change if the laboratory environment was changed in regards to the absence and presence of light and temperature changes. A possible study design would be to change the light and temperature at which the *E. japonicus* is growing under. For example, red light and a colder room temperature could be slowly introduced to one group and compared to one grown in the shade under room temperature and one in the presence of sunlight and room temperature. By following such a study design, we can see the variation in phenotypic selection on temperature and absence or presence of sunlight. Additionally, one can ask if phenotypic plasticity reversible, if the plants grown under red light and cold temperature over several generations adapt to room temperature and sunlight if the temperature and light are changed overtime.

FIGURES AND TABLES

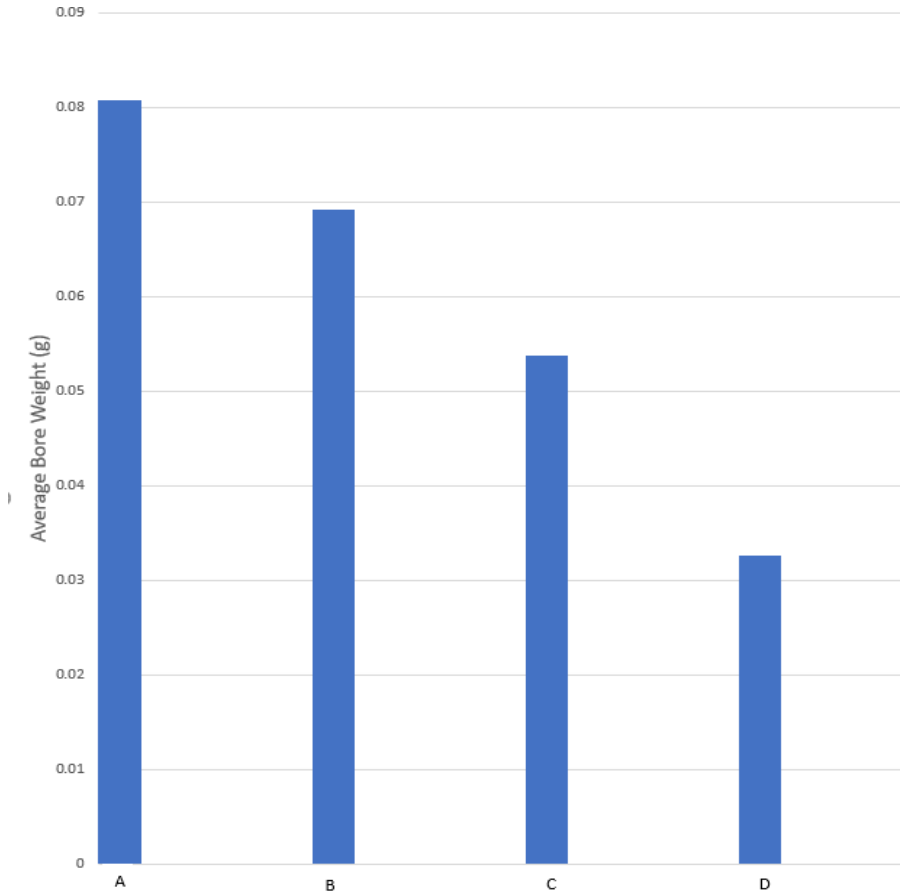

Figure 1. Average bore weight (g) of *Euonymous japonicus* and *Vincetoxicum rossicum* under sun and shade conditions for the 2019 growing seaon. A= *E. japonicus* in the presence of light; B= *E. japonicus* in the absence of light; C= *V. rossicum* in the presence of light; D= *V. rossicum* in the absence of light

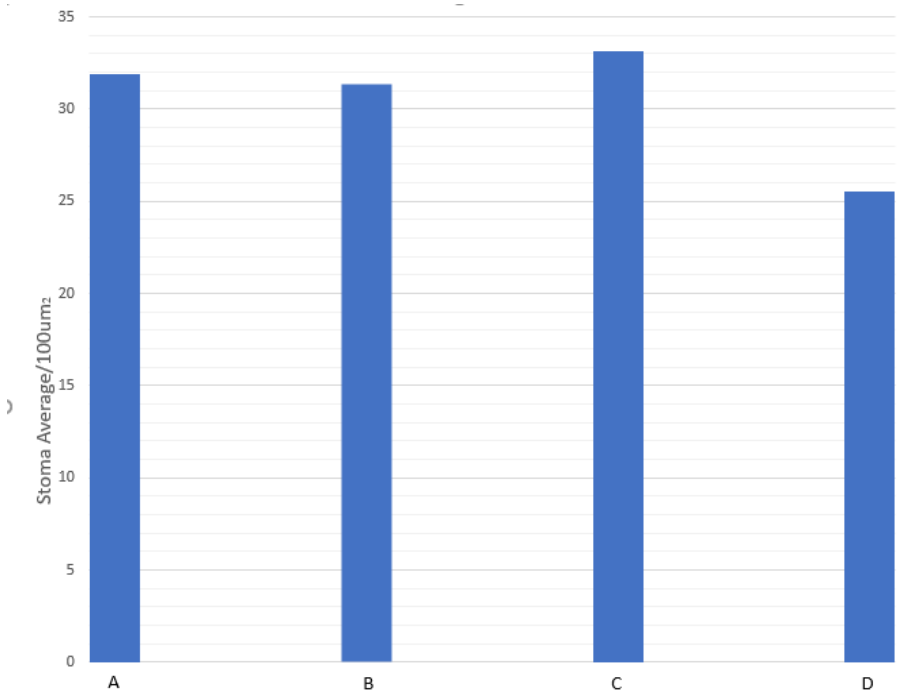

Figure 2. Stoma average/100 um2 of *Eunonymous japonicus* and *Vincetoxicum rossicum* under sun and shade conditions for the 2019 growing season.
A= *E. japonicus* in the presence of light; B= *E. japonicus* in the absence of light;
C= *V. rossicum* in the presence of light; D= *V. rossicum* in the absence of light

E. japonicus		*V. rossicum*	
SUN	SHADE	SUN	SHADE
0.454	0.397	0.653	0.417

Table 1. Average absorbance at 652nm of *Euonymus japonicus* and *Vincetoxicum roossicum* under sun and shade conditions for the 2019 growing season.

LITERATURE CITED

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