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Dogbane Under Stress: Habitat Differentiation of Anthocyanins in Apocynum cannabinum

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Photo by: Chris Wolfe

Photo by: Brittney Way

Introduction:

The quarry in DePauw's Nature Park, which was previously stripped of soil and layers of bedrock, offers an ideal study system for understanding how plants adapt to disturbances. The quarry bottom is particularly stressful for plants because of this loss in soil, high variability in water availability due to the poor porosity of the bedrock, and high light intensity. Therefore, plants that live there must have traits suitable for this harsh environment. One response elicited to stress in plants is the production of red pigments called anthocyanins. Anthocyanins are versatile and have been found to be protective in a multitude of ways; among their many functions, they have been shown to protect against tissue damage at high light levels and increase herbivore resistance. Using Hemp Dogbane, *Apocynum cannabinum*, as a study system, we asked whether anthocyanin content in stems differs between quarry and non quarry environments, if there is a relationship between pigmentation and herbivory, and how pigmentation is selected on in the two habitats.

Methods:

Using preliminary data on anthocyanin concentrations in six populations, three inside and three outside the quarry bottom, we determined that the sites Quarry Meadow and Quarry One were the most differentiated in their respective concentrations. These two populations were revisited in mid-July and censused for herbivory and fitness parameters. Using the total number of leaves, total damaged leaves, and average percent damage, we calculated the total damage across each plant. The number of fruits and their respective lengths were measured for fitness estimates. Using stem tissue below the terminal bud, anthocyanins were extracted and analyzed using a spectrometer.

Site Description:

The three quarry bottom populations (Q1, Q2, and Q3) experienced similar stressful environmental conditions with minimal soil and harsh sunlight. All three populations faced intermittent periods of flooding, although Q3 faced the most. The three non quarry sites, Quarry Meadow, Prindle, and Rim1 had much more variation between populations due to differences in vegetation, light and water levels. Quarry Meadow, located just outside the quarry bottom, was inundated with water for parts of the summer and had open, primarily nonwoody, vegetation. The Prindle site more closely resembled a prairie, having more direct sun exposure. Rim1, located on the western side of the quarry nearby R5, was exposed on the edge of the quarry wall and individuals grew atop a rocky surface.

Results

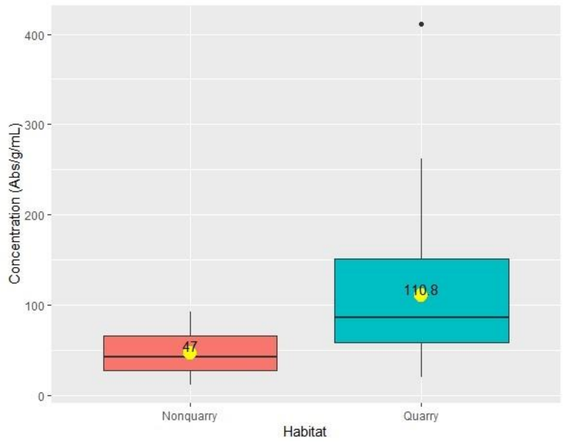


Figure 1: Boxplot showing the difference in anthocyanin concentration between non quarry (red) and quarry (blue) habitats. The mean concentration (Abs/g/mL) of the quarry meadow population was 46.98 whereas quarry one was 110.76. After log transforming the data, the p-value of the t-test between both environments was < 0.0001.

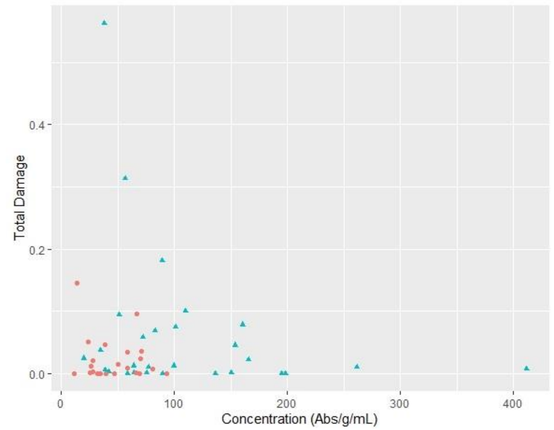
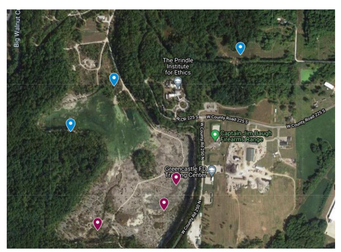


Figure 2: Scatterplot plotting concentration of anthocyanin and total damage of individuals for quarry (red) and non quarry (blue) environments. No significant relationship between anthocyanin concentration and herbivory was found across both populations and within each.



Map showing all six populations. Blue are non quarry sites, red are quarry sites.

Cross section of Dogbane stem under SEM. Anthocyanins were analyzed on the epidermis, or outer layer, of the stem.

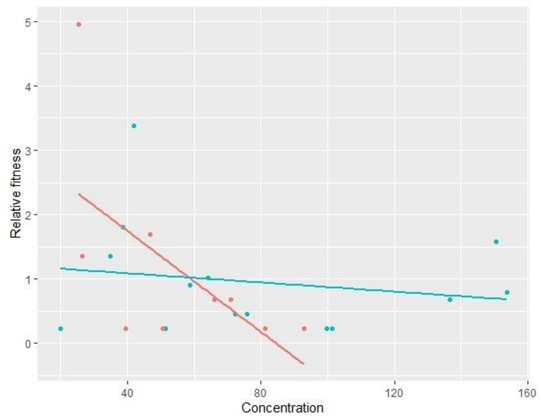


Figure 3: Graph showing the selection on anthocyanins for both non quarry (red) and quarry (blue) environments. Relative fitness was calculated by taking individual fruit number divided by average fruit number of both populations. The concentration of anthocyanins was a significant predictor of relative fitness (p=0.022), and there was marginally significant interaction between concentration and habitat (p=0.052) visualized by the difference in best-fit lines.

Conclusion:

There is a sizable difference in anthocyanin production in the quarry and non quarry environments, with the quarry having a mean concentration more than twice that of the non quarry population. Two mechanisms, plasticity and local adaptation, could be behind these stark differences and it is plausible that both are affecting this differentiation. If local adaptation is occurring, there would a change in allele frequency in favor of the optimum level of anthocyanins in each environment. However, phenotypic plasticity may be a more likely cause of differentiation in the quarry given the amount of time needed for selection to occur in populations.

Light levels have been shown to influence anthocyanin production in dogbane and it is likely that the high influx of light in Quarry One is prompting dogbane to produce more of these photo-protective pigments. However, many other factors that were not studied, such as heavy metal concentration and nutrient availability, may also be promoting these quarry plants to produce these pigments.

Although it was hypothesized that anthocyanins could protect against herbivory, our data does not support this claim. When populations were analyzed separately for herbivory, herbivory levels were not affected by concentration of anthocyanins. Specialist species located in the Nature Park could be adapted to potential chemical defenses in anthocyanins.

Surprisingly, concentration of anthocyanins was a significant predictor of fitness, however not in the direction originally anticipated: higher concentrations led to lower fitness. A third correlated factor, such as stress in the quarry, could also be affecting fitness and anthocyanin production, contributing to this unanticipated inverse relationship.

Future Recommendations:

Future research could involve cross transplanting individuals from both populations in order to gain closer insights into the role of plasticity in anthocyanin production.

References:

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