Introduction

This summer we, Jacob Katz, Knut Vanderbush, and Obioha Chijioke, continued our work at the Lisman Laboratory for Molecular Ecology at Riverdale Country School under the guidance of Dr. Rachel Cox. Through our three-year study of *Picea* in the Brooks Range, Alaska and Black Rock Forest, New York, we sought to determine the epigenetic response of *Picea* to environmental stress along a latitudinal transect. In order to further establish the methylation trends we observed in *Picea* and improve our methodology for epigenetic research, we collected an additional set of samples in 2019, all from the nearby Black Rock Forest. We also continued our analysis on frozen DNA from samples taken from Alaska and New York in 2018. Field and lab research took place between June 14th and July 19th. Data analysis continued through early December.

Summary of Findings

Our recent findings can be divided into two categories. The first is our methodology analysis, which began in 2018 with an inquiry into the variation in methylation between embryonic and mature needles taken from the same branch. Through research in Black Rock Forest in 2019, this portion of our research expanded to include an analysis of the variability of global methylation in a more stressed *Picea* population compared to that of a more favorable population. Our methodology research also sought to determine the impact on methylation of our current procedure for storing DNA. These methodology studies have important implications for the future of our research and other epigenetic research in related species. Notably, they have also provided insight into the variability of methylation levels within an individual or population.

The methodology study sought to answer three fundamental questions:

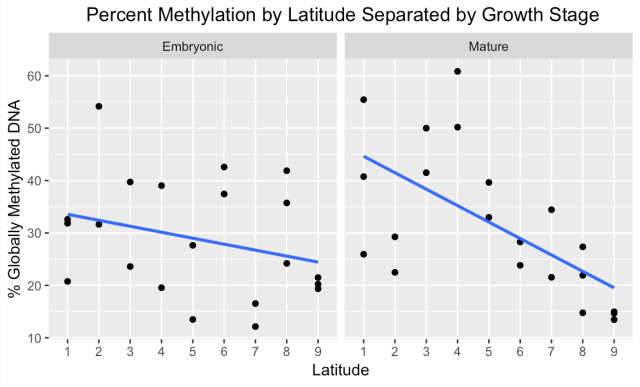
1. Do embryonic needles experience greater variability in methylation levels between samples than mature needles from the same population?
2. Do populations with greater overall stress exhibit higher variability in methylation levels between individuals?
3. Does a year in a -20C freezer change the methylation of *Picea* DNA and, if so, does methylation change at a consistent, observable ratio?

The second major category contains data from our work since 2017 on latitudinal stress and competition stress data from Black Rock Forest in 2019. Our previous data showed increased methylation at higher latitudes, where increased exposure would subject trees to more harsh environmental conditions such as winter winds. This year our analysis on *Picea* in Black Rock Forest showed a similar increased methylation in trees with competition related stress. Collection

in Black Rock Forest was originally meant only to help test methods questions and provide an important base for our latitude/treeline study. This year we were surprised to observe that the two groups of *Picea* trees we study in Black Rock Forest were living under notably different conditions. We believe one group of trees experiences significantly greater competition for natural resources than the other. This is also the leading hypothesis of Dr. Kevin Griffin from Columbia University, who, based on our molecular analysis, is interested in setting up the ambient monitors he uses in Alaska on those trees. Our findings are represented below.

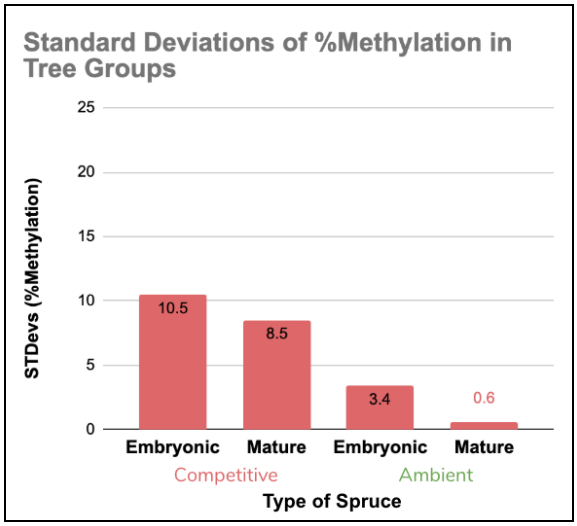
Specific Findings - Methodology

1. Embryonic and Mature deviation



This graph displays our measured methylation levels for all 2018 samples, where latitude 1 is the northernmost edge of treeline, latitudes 2-8 are along a latitudinal transect within the Boreal Forest, AK, and latitude 9 represents trees from Black Rock Forest. The data is split between the mature growth samples and the embryonic growth samples from the same individuals. Although methylation is highly variable in both cases, the data for mature growth displays a (statistically significant) trend of increasing methylation with higher latitudes, while the data for embryonic growth is not strong enough to confirm such a trend. We believe that the important role of methylation in controlling embryonic development that has been found in many species causes embryonic growth within a group of trees to exhibit great enough variability to mask the effects of environmental stress response methylation.

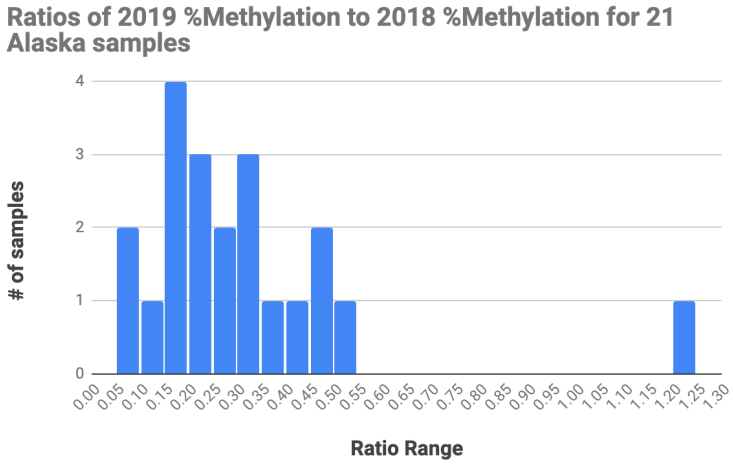
2. Variability in high-stress populations



Our 2019 methylation findings in two different Black Rock Forest populations corroborate the idea that embryonic growth populations exhibit increased variability: in both populations, the standard deviation of methylation levels between all individuals in the population was greater in embryonic growth. In fact, we would expect methylation in embryonic growth to be even more variable, but this year, though we collected our samples in mid-June like in the past, spring came earlier and we found our samples later in the trees' growth cycle. By this point, their embryonic growth was observationally and quantitatively closer to mature, which could explain the lower standard deviations.

Furthermore, in both embryonic and mature growth, trees from the more competitive population, which was under greater stress, exhibited greater variability in methylation than their favorable "ambient" counterparts. Therefore, it may be harder to determine specific average methylation values in populations under stress. This could explain why it was so difficult to find tight methylation trends in 2018, where our Alaska samples would be under far greater stress than the competitive Black Rock Forest samples. Interestingly enough, the limited data from Black Rock Forest (latitude 9) represented on the scatter plot above show a very low standard deviation as compared with Alaska samples.

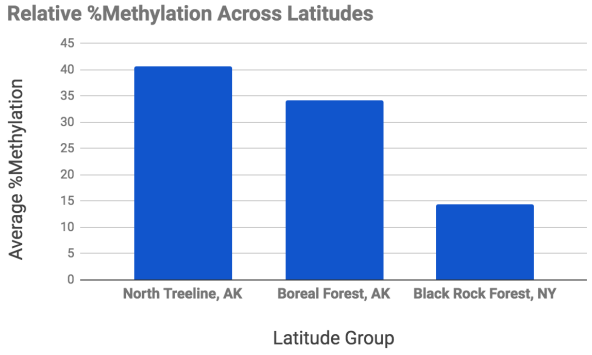
3. Freezer Storage deviation



After deriving methylation levels again in 2019 for 2018 samples that had been stored in a freezer for a year and for which we already had data, we calculated the ratio in methylation for each sample between 2019 and 2018, then plotted those ratios on a histogram. Most methylation levels decreased during a year in the freezer by anywhere from 50% to 95%. With such a variation, we were not able to determine a precise strategy to convert a frozen sample's methylation level into the methylation level it would have had when we first collected it, which is when we would have wanted to analyze it. In the future, we must analyze all our Alaska samples the year we collect them.

Specific Findings - Stress Response

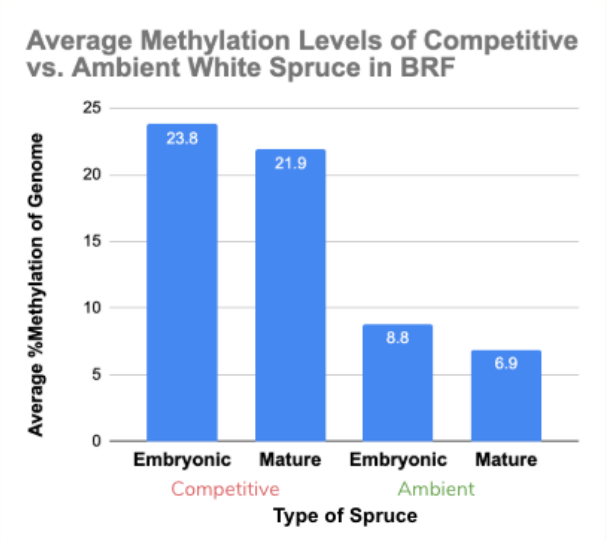
1. Latitudinal trend



Our 2018 findings indicate that, on average, trees at the northern edge of the treeline, which are extremely stressed, exhibit greater methylation values than trees in the less stressful Boreal Forest or the verdant and comfortable Black Rock Forest. This suggests that *Picea* in more

stressed populations exhibit greater methylation. This graph only uses mature growth data, since the embryonic growth data was too variable to find a trend.

2. Competitive vs ambient



Corroborating our 2018 findings, our 2019 findings show that, in Black Rock Forest, trees in the competitive population display much greater methylation on average than trees in the less stressed ambient population.

We were initially surprised to find that the trend was just as noticeable in embryonic growth as in mature growth, but we concluded that this was likely due to collecting samples at a later point in the trees' growth cycle as described in part 2 of our methodology results. The graph in that section also indicates the standard deviations associated with these means.

Conclusion

Our overall findings in terms of methylation levels across different populations inform us about the nature of the epigenetic stress response. Our data implies that *Picea* under greater stress will shut down all but their necessary genes to survive rather than opening up their genomes to new survival tactics.

Our methodology findings also carry important information about the epigenetic stress response. The fact that methylation was more variable within embryonic growth corroborates recent findings that epigenetic modifications are used to regulate other vital processes in embryonic growth apart from the stress response. The trend that methylation was more variable in populations under greater stress suggests that individuals in those populations make a wide range

of “trial and error” attempts to respond to stress through epigenetics (although their methylation is higher on average).

In addition, our methodology findings inform the reliability of our various trends in different environments given other sources of variability. For instance, there was no clear trend across latitudes in embryonic growth in Alaska, because that data is taken both from embryonic growth and from a stressful environment overall, but there was a clear trend across populations in the overall safer Black Rock Forest within the embryonic growth which we believed was closer to mature. Furthermore, disturbing methylation levels by freezing samples for a year would introduce another layer of variability.

We would like to once again extend our sincere gratitude to the Marjot Foundation for supporting our research. Climate-oriented ecological research has never been more urgent.