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**Discovering the Genetic Diversity and Cold Tolerance of *Aphis nerii***

**Introduction:**

My FURSCA research that I conducted this summer was on The Genetic Diversity of *Aphis nerii. Aphis nerii* (milkweed Aphids) are a recurrent invasive species that are well known as pests of cultivated plants. These insects are fascinating because they reproduce asexually, giving rise to low genetic diversity. Because the insects have low genetic diversity, they are unable to overwinter; asexual reproduction does not allow the aphids' eggs to be cold-resistant compared to sexual reproduction, causing their inability to overwinter (Simon et al. 2002). This is why they successfully reproduce in warm temperatures. For my project, I conducted two separate experiments. The goal of my project was to determine the presence of low or high genetic diversity in *Aphis nerii* to better gain an understanding of how the aphids are able to adapt to their environment despite predominantly reproducing by asexual reproduction and having low genetic diversity.

In addition to the second experiment, I wanted to determine the cold tolerance that aphid populations have in various conditions. This experiment would allow me to know the range of temperatures that the aphids could withstand. Knowing this relates to the prediction of genetic diversity because it may showcase that not all of the populations are genetically similar and are instead adapting or evolving for their environment.

**Methodology:**

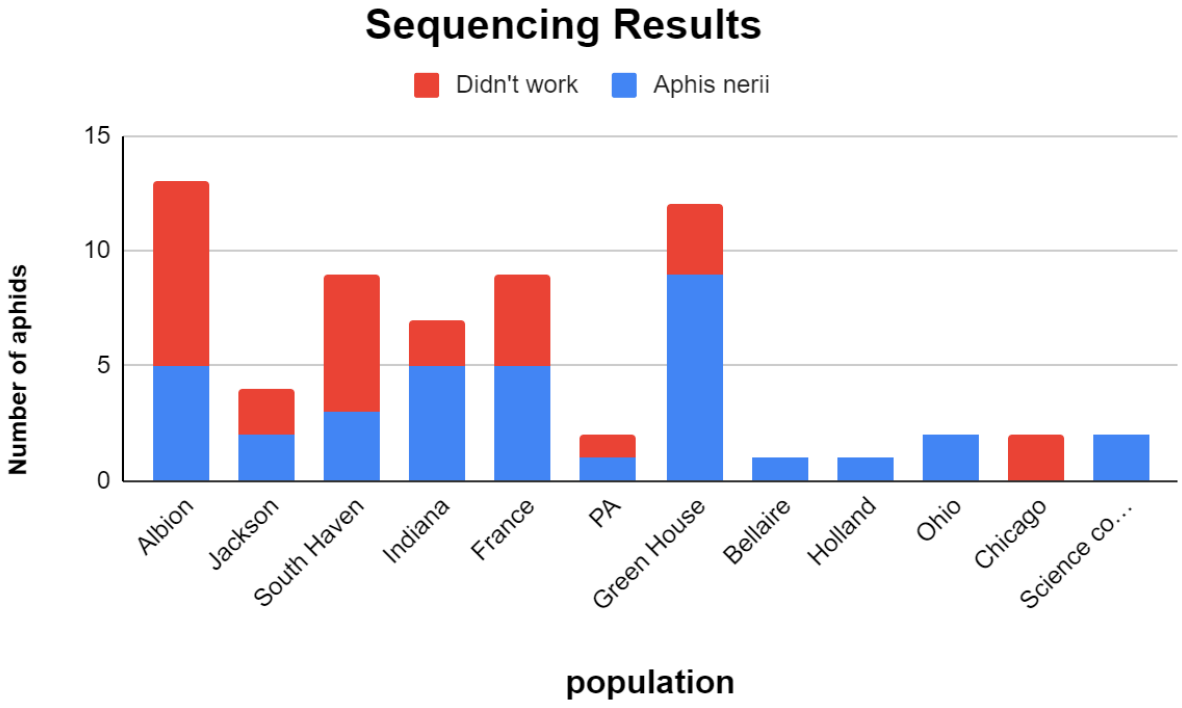
For the first three weeks of FURSCA, I focused my attention on my first experiment. For my first experiment, I performed various techniques to acquire DNA from various samples collected from myself, Dr. Cahill, and previous students who have worked on this project. These samples were collected from the United States in different states and cities, including the species' native region, France. For each sample, I performed the following techniques in a specific order: 1. lysis; 2. DNA extraction; 3. electrophoresis; 4. PCR; and 5. Electrophoresis again. After successfully obtaining the material I needed, I sent the DNA to the Michigan State University Sequencing Center to receive the specific DNA sequences of each sample or aphid I shipped off.

While I was waiting for my sequences, I conducted my second experiment: The Cold Tolerance of Milkweed Aphids. For this experiment, I collected milkweed aphids and Dogwoof Aphids (another species) from outside of the science complex at Albion College. For the first trial, I only tested the Dogwood aphids by placing about 20+ aphids in four separate glass cylinders. Each cylinder was labeled with different times (15 mins, 20 mins, 25 mins, and 30 mins). I placed each cylinder in the lab's fridge at -10°C at the indicated times above; after the time of the cylinder was up, I placed them at room temperature and recorded the mortality rate of the aphids after about three hours. This is important because, like many other insects, in cold temperatures, aphids tend to become less mobile and can appear to be deceased. I will proceed with touching each one to determine if they are still alive. This process will ensure that the mortality rate is recorded and determined accurately. For the second trial, I tested this same

method on the Milkweed Aphids and another species of brown aphid that I was unable to identify. This consisted of four separate cylinders of Milkweed aphids and four other separate cylinders with black aphids. However, instead of the times ranging from 15 minutes to 30 minutes, they were 30 minutes, 60 minutes, 90 minutes, and 120 minutes. I increased the times because the Dogwood aphids in the first trial survived in all of them.

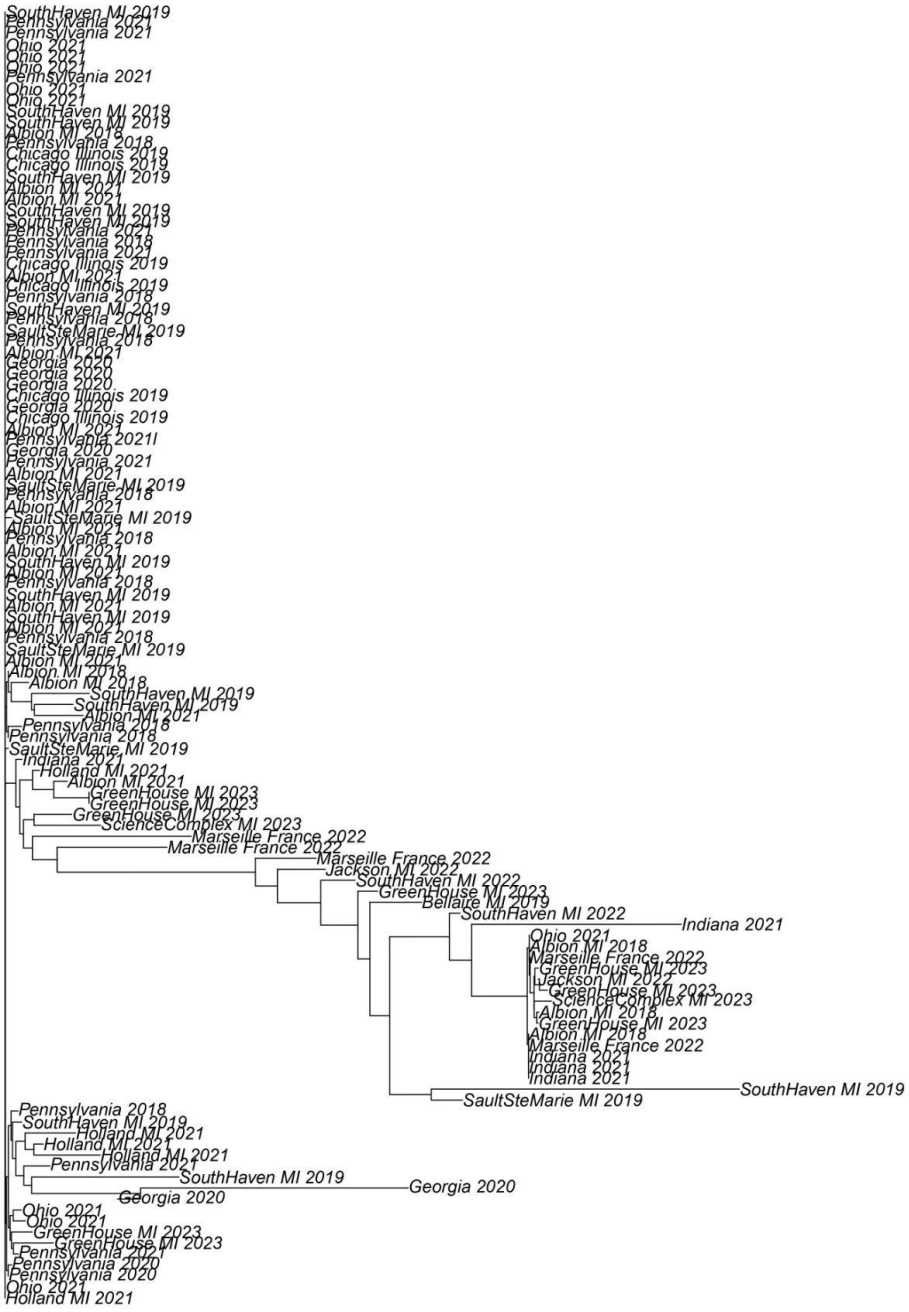
**Results:**

For the first experiment, after receiving the sequences from MSU, I identified the species of each sequence using BLAST, a common online tool in bioinformatics. After identifying each sequence, I ruled out the sequences that were *Aphis nerii* vs. other species'.



## The Sequencing Results of Each Sample

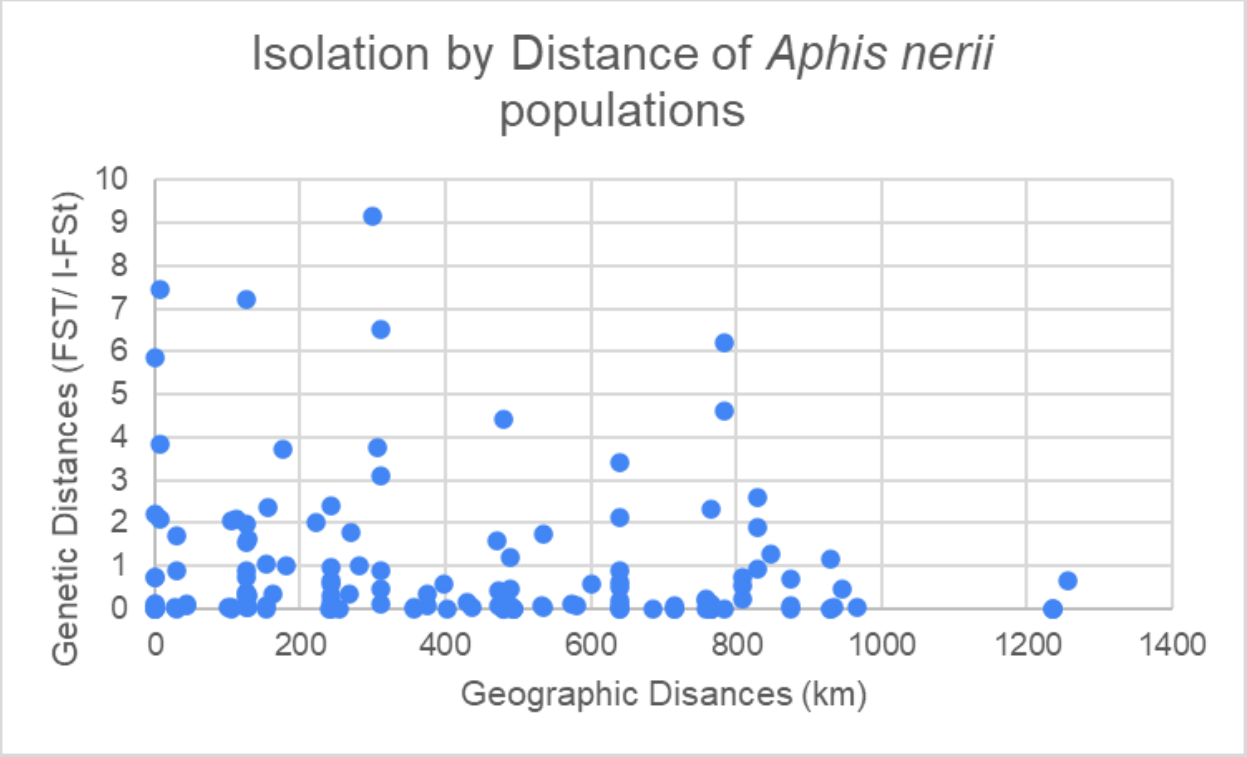
In total, I had sampled and shipped 63 samples, but only 36 of those were *Aphis nerii.* As shown above, the blue indicated in the graph is the *Aphis Nerii* sequences that were identified in each population that I sampled, and the red indicates the sequences that were not *Aphis nerii.* This information is vital because I wanted to make sure that I was only analyzing *Aphis nerii,* as they are the species that reproduces asexually and has the lowest genetic diversity; other aphid species' do reproduce sexually and therefore have a greater genetic diversity, so having that included would screw up the results of my data analysis.




**Phylogenetic Tree of Aphis Nerii**

By using Rstudio (A data analysis program), I was able to create a phylogenetic tree of the samples that I worked with, including those of previous students, making a total of 117 aphids on the tree. This tree allowed me to view how genetically similar each aphid of a particular

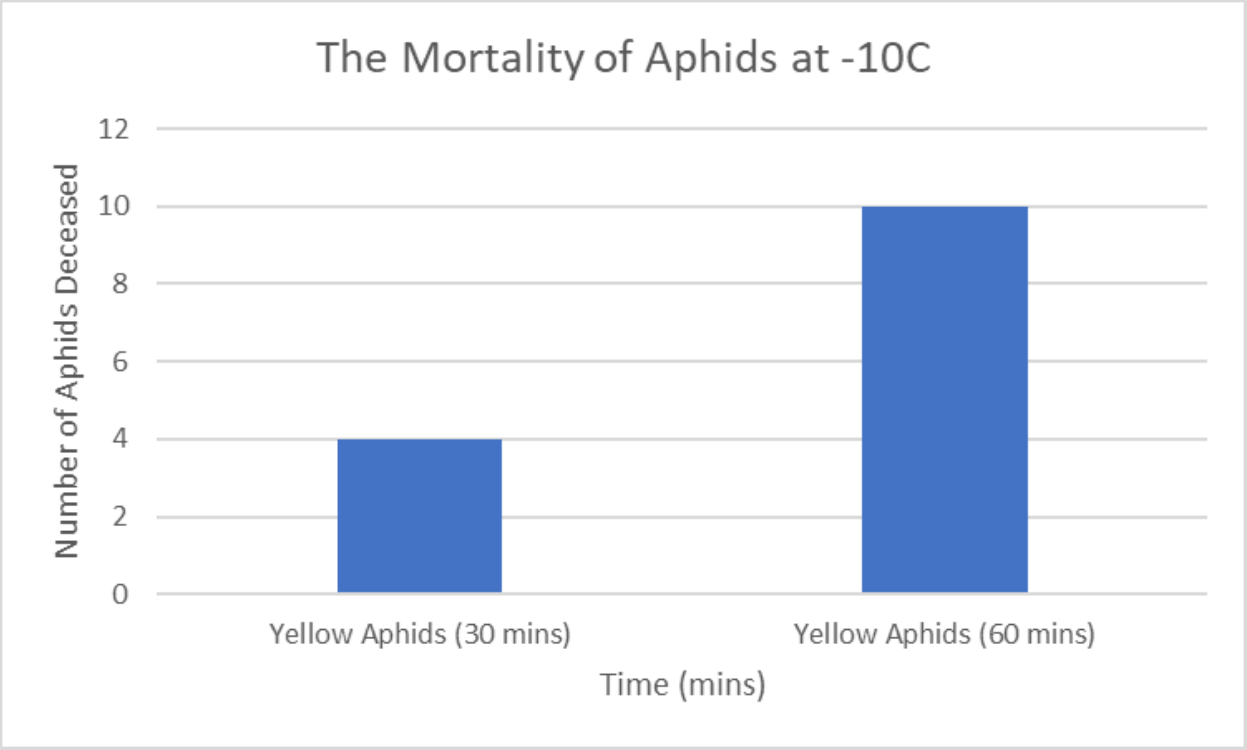
population and year was to other aphids collected. When viewing the top half of the tree, the populations that are clustered together are more genetically similar than those in the middle or bottom of the tree. As a branching off occurs, this indicates that genetic differentiation is occurring. I found it intriguing that some of the aphids from the same year or population were very genetically different but more genetically similar to other populations, some even being over one thousand miles apart. For instance, in the middle of the tree, there are samples from Marseille, France, that are very genetically similar to an aphid from Jackson, Michigan. But, when comparing the other Marseille samples, they are far apart, showcasing that even though those aphids were collected together from the same population, they are more genetically different than an aphid on a different continent.




**Graph Showcasing if Geographical Distance Affects Genetic Diversity**

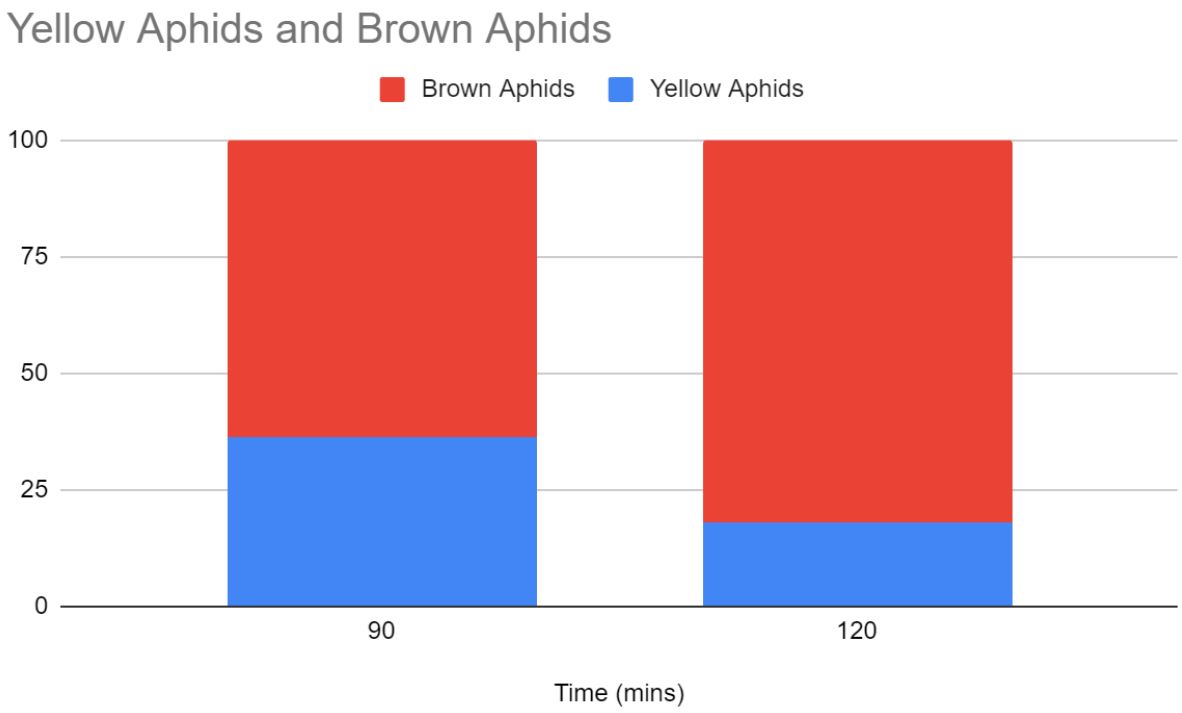
I used Google Earth to measure the distances between each location where the aphids were sampled and Rstudio to view the specific genetic diversity of each sample (FST/I-FSt). France was excluded because it is the aphid's native range and on another continent, and would therefore be the outlier in the data. It was revealed that there was no linear relationship between geographical distances and genetic diversity.

**Experiment 2: Cold Tolerance**




**The Cold Tolerance of *Aphis nerii***

At 30 minutes, there was a very low rate of dead yellow aphids; however, as shown above, at an hour, half of the aphids in the 60-minute group were dead. This indicated to me how long the Milkweed aphids (yellow aphids) could withstand the cold temperature.




**The Mortality Rate of Brown and Yellow Aphids at -10C**

Continuing the analysis, I compared the mortality rates of the brown aphis and yellow aphids at 90 and 120 minutes. The brown aphids had more deaths than the yellow aphids, but when focusing on the goal experiment, there was a significant difference in deaths due to the longer periods of time.

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**Discussion:**

The goal of my project was to determine the presence of low or high genetic diversity in *Aphis nerii* from various populations to better understand how they are adapting to their environment. And determine the cold tolerance of *Aphis nerii.* Based on my data, I was able to accept my hypothesis and conclude that low genetic diversity was present despite geographical

differences, corresponding with previous studies. However, the diversity was not as low as I expected; my phylogenetic tree revealed that the samples were not identical and contained differentiation, which indicates that possibly mutations are occurring in some of these populations, causing some aphids to be genetically different from others, and possibly in the far future, there could be evolution occurring.

In addition, I was not able to find the cold tolerance of *Aphis nerii* due to a lack of resources and limitations on time. Yet, my experiment revealed that the Milkweed Aphids have a difficult time being in cold temperatures for a long time. For more concrete results, I would like to have more sources of colder environments in the future.

**Conclusion:**

FURSCA allowed me to gain experience conducting my own experiments and, of course, participating in research. I was able to learn valuable skills such as data analysis using code, how to manage in a short amount of time, and how to persevere when experiencing setbacks because research is never perfect. I believe my technique and journey were smooth and enjoyable for the majority of my time because of my advisor, Dr. Cahill, who allowed me to push myself and make decisions for my project. I would also like to thank the previous students who worked on this project in the past, Irene Corona Avila, Katie Ferrero, Brian Lomeli Garcia, and Patrick Mayo, who collected and also sampled the aphids, which was a major component of my project. And finally, Renee Kreger Renee Kreger and Elizaberh Palmer, for supporting me to make sure my stay on campus was comfortable. In the future, I plan to present my research at the Elkin Isaac Symposium and the SICB conference.

Citations

Harrison, John Scott, and Edward B. Mondor. “Evidence for an Invasive Aphid ‘Superclone’: Extremely Low Genetic Diversity in Oleander Aphid (Aphis Nerii) Populations in the Southern United States.” *PLoS ONE*, vol. 6, no. 3, 2011, https://doi.org/10.1371/journal.pone.0017524.

Purkart, Adrián, et al. Citizen Science Reveals the Current Distribution of the New Plant Pest Aphis Nerii in Slovakia. *Plant Protection Science*, vol. 56, no. 2, 2020, pp. 101–106., https://doi.org/10.17221/46/2019-pps.

Simon, Jean Christophe, et al. Ecology and evolution of sex in aphids *Trends in Ecology & Evolution,* Vol.17, No.1, 3 Jan 2002, https://www.sciencedirect.com/science/article/pii/S016953470102331X.