

# <u>Central Park Pond Phenology Trail - Multiple Individuals</u>

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## Abstract

This study continued to monitor the phenology trail established around the Pond in Central Park in 2018 by discerning physical changes in twelve plant species over eight months and using DNA barcoding to confirm our visual identifications of the species. It also monitored multiple individuals of each species and collected temperature data both inside and outside of the park. The gathered DNA barcodes were isolated from leaf samples by performing DNA extraction, PCR and gel electrophoresis before they were sent off to Genewiz for sequencing. We hypothesize that the phenophases of our plants will differ both from typical trends and from others in their species due to the urban heat island effect. Our results showed that expressed phenophases are not always constant within a single species and that the urban heat island effect had an impact on the observed phenophases.

## Introduction

Phenology is the study of the impact that environmental changes have on organisms, especially in their appearances, their interactions with their environment, and their method of reproduction (1). In plants, these changes, also known as phenophases, can result from a variety of different factors, such as the temperature of their environment, which can impact the change from vegetative to reproductive growth, or the quantity, quality, and duration of light to which they are exposed, which can help control both the amount of growth over time and the timing of flower opening (1, 2, 3). Additional factors that can have an impact on a plant's phenophases include the availability of water and nutrients in the soil and the humidity of their environment (3). The timing of these changes impact a plant's growing season, defined here as the time between the first flowering time (FFT) or the leaf out date (LOD) and the first leaf fall (LFA) (4). This timing is measured with a phenophase calendar, which shows phenophase changes in individual species over time, which can then be compared to typical trends for each species (5). The results obtained from phenological studies can be used by biologists to observe the impacts of both organic and inorganic environmental changes on the natural world. Phenology is also useful for citizen science projects, allowing non-scientists to observe and document the same organism over long periods of time. Phenology trails serve as a collection of specific organisms within one area that can be observed repeatedly to discern changes in any aspect of their appearance and behavior (1).

Due to Central Park being located within a metropolitan area, it is subject to a 1 to 3 degree celsius temperature increase when compared to surrounding rural areas due to microclimates created by the urban heat island effect (6, 7). The urban heat island effect results from an increase in heat energy in metropolitan areas compared to rural areas due to the high concentration of people, cars, and buildings in cities (6). Therefore, we predict that the phenophases of the plants within the phenology trail will vary both from other individuals of their species and from typical trends due to the warmer temperatures, although the impact of this effect will vary based on the species and on what factors impact their phenophase changes.

This project utilized the phenology trail previously established around The Pond in Central Park to collect data on the phenology of multiple different individuals of the twelve plant species on the trail (5). We aimed to determine if the differences in phenophases from typical trends observed in the earlier study setting up the trail are constant across multiple individuals of each species and over multiple years. In addition, we hoped to confirm the identity of the plants on the trail through DNA barcoding and to accurately collect temperature data both inside and outside the park in order to determine the impact of the urban heat island effect.

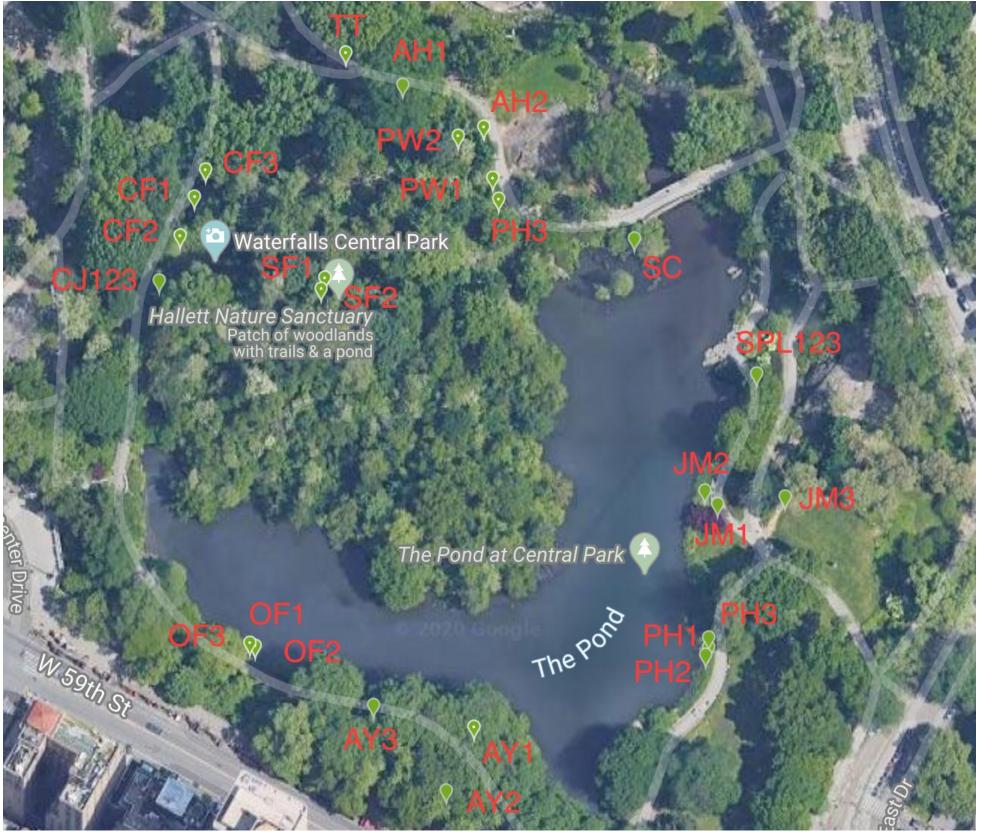


Figure 1: Map of the phenology trail.

# Materials & Methods

Two to three individuals of twelve plant species were selected along the trail surrounding the Central Park Pond, New York. The species were chosen based on their location and in order to have representatives of many different plant families. After being chosen, all individuals of each species were visually identified and confirmed through iNaturalist with the help of scientists on the platform. Each plant individual was monitored as often as possible over a period of eight months, from October to May. The plants were monitored in weekly observation sessions in the park. Each observation period consisted of measuring the height, width/diameter (taken at the widest part of each plant, including foliage), leaf number, growth stage, and any additional observations. Individuals were photographed, and the data was recorded within the iNaturalist project "Central Park Pond Phenology Trail." This data was then compared between individuals and with typical phenophases of the species, which were determined through data from outside researchers of various universities or organizations, in order to see how the phenophases of the monitored plants differed both from each other and from typical trends. Physical samples of the plants were collected with permission from the Parks Department. These samples were collected by placing a sample tube around the tip of a leaf from each individual of each species and closing the tube on the leaf, the pressure of which sniped the tip off and caused it to fall into the tube. The samples were refrigerated in the science lab at the Browning School.

Temperature data both within and outside of the park was collected by use of a PocketLab<sup>TM</sup>. After being charged completely, the PocketLabs<sup>TM</sup> were connected to the accompanying app on an iPhone through a wireless connection and placed on a lanyard so that they could record the outside temperature without our body heat interfering with the measurements while holding them. At each site, the PocketLab<sup>TM</sup> was checked to ensure it was turned on and recording data before checking the app, which showed the temperature levels in real-time at each site. When a temperature reading stayed at the same level for at least five distinct readings, the reading was recorded. Temperature readings from the PocketLabs<sup>TM</sup> were recorded at each site in the park and at a single site outside the park on Madison Avenue. In addition, the temperature of the Pond water was determined using a digital thermometer, which was directly inserted into the water and held there for a few minutes until it acclimated to the temperature of the water and was able to provide an accurate readout of the water temperature. The average temperatures within Central Park over the given months were collected from past averages obtained by the National Weather Service. Average temperature data from outside of the city was collected through the use of WeatherUnderground<sup>TM</sup> weather station data from the rural area of Latham, New York

DNA extraction and amplification was performed by following the Barcoding protocol outlined in the DNA Learning Center procedure. We used the Ready To Go PCR beads and *rbcL* primers were utilized during the PCR process. The *rbc*L primers were chosen because the *rbcL* gene is found in the chloroplasts of most photosynthetic organisms, which includes the plants in this study. A few modifications were made to the DNA Learning Center procedure, including beginning heating up the incubator for the initial incubation period during the early steps of extraction so that it would be warm enough to immediately insert our samples into it and keeping the samples in the incubator for fifteen minutes rather than ten minutes during the first incubation period in order to increase DNA yield (8). These samples were then loaded into a gel electrophoresis machine, and the resulting gel signatures were analyzed in order to confirm the success of the extraction and amplification before being sent to GeneWiz for sequencing. We used the BLAST process on DNA Subway to identify the species.

Level of leafing of a typical rural pokeweed and a Central Park pokeweed

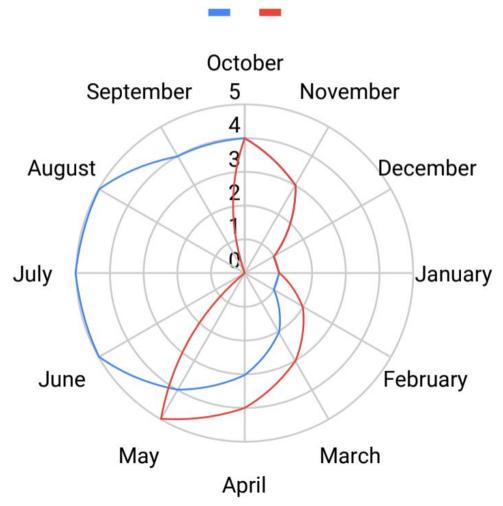
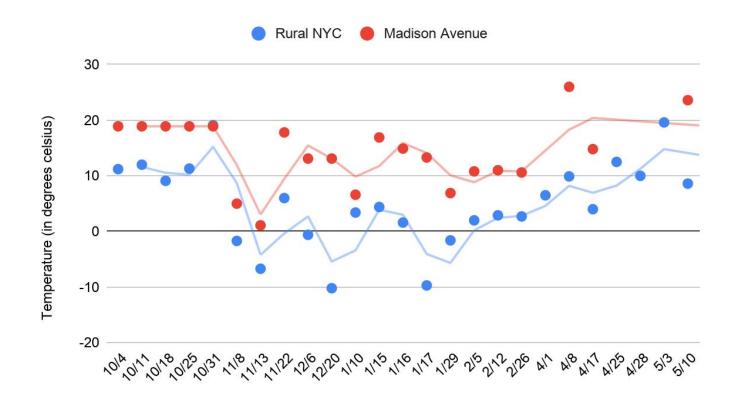


Figure 2: Circular graph showing leafing phenophase variance and typical leafing phenophases in one of our species, the American pokeweed (Red line is the monitored pokeweed, blue line is the typical pokeweed).

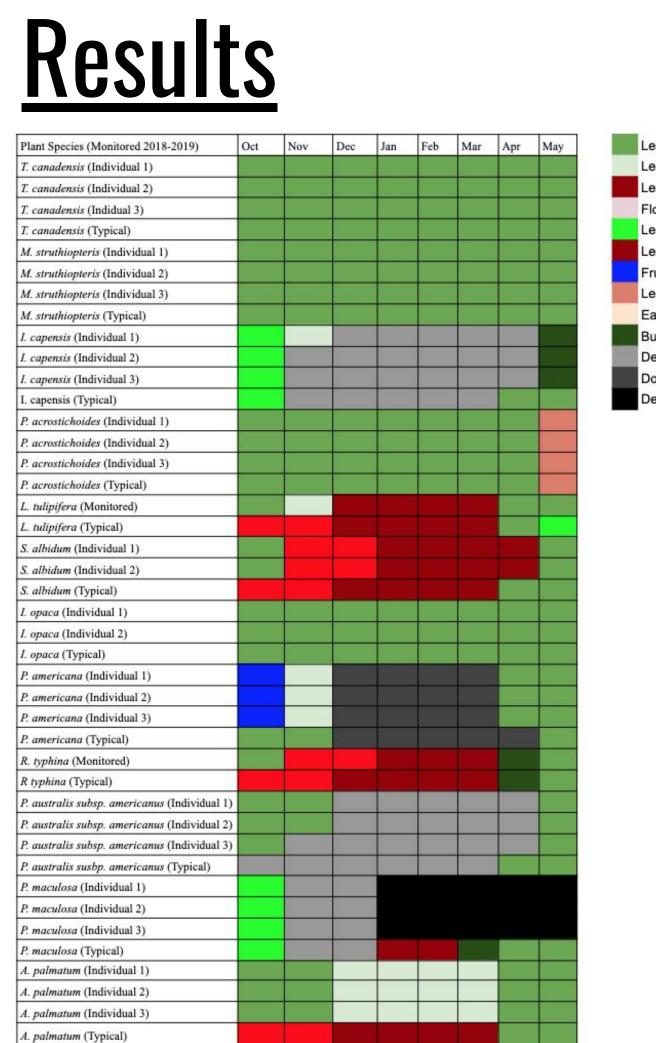


**Figure 5:** Temperature fluctuation graphs from October 2019 to May 2020 for New York City and Latham, New York.





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The results of this study support both our hypothesis that the phenophases of our study plants would vary due to the urban heat island effect and our hypothesis that there would be noticeable differences between the phenophases exhibited by different individuals of the same species. Although the phenophases of some of our plants, such as the American yew and the American holly, did match up with typical trends, other species, such as the American pokeweed and the American common reed, did show noticeable phenophase differences in regards to timing of regrowth and timing of decay respectively.

In addition, while the phenophases of some species, such as the ostrich fern, were constant across all individuals, those of others, such as the sassafras and the American common reed, had noticeable phenophase differences between individuals, in regards to earlier regrowth in one individual compared to the others and later decay in one individual compared to the others respectively. Interestingly, the Christmas fern, which did show variation in our previous study, showed no sign of variation in this study and the sassafras, which did not vary from typical trends in the previous study, showed noticeable phenophase differences in this study, suggesting that individual variation can cause the phenophases expressed by the same individual to differ from year to year.

Some sampling errors did occur during this study, as the PocketLabs<sup>TM</sup> occasionally didn't function correctly, preventing us from collecting temperature data on certain days. In addition, due to a sequencing error that may have resulted from either poor quality sequences or added distilled water, the samples of the second individual of the American yew, the first individual of the Japanese maple, the second individual of the American common reed, and the first and second individuals of the common jewelweed were misidentified. Moreover, we were prevented from performing the DNA barcoding process on the majority of our samples as a result of the COVID-19 pandemic, which, combined with our sequencing errors, meant that we had to rely on visual means to identify the individuals of each species.

The results of this study are significant because they provide further evidence on the impact of the urban heat island effect on the natural world. This data may prove helpful in future environmental decision-making, as it can be used to help determine the impact of the temperature increase and other effects of urbanization on New York City's plantlife, which may help ensure its survival in an ever-warming world. In addition, the information obtained through this study, along with all past and future research on this trail, will continue to be used by both researchers and citizen scientists to observe the phenophase changes on the trail long after the end of this study.

As the phenology trail utilized in this study will remain online and accessible to both researchers and citizen scientists, there are many possible future studies that can follow off of this project and its findings. One option for a potential future study would be a year-round study of the individuals on this trail, as this study did not collect any data from the summer months and lost several monitoring days as a result of the COVID-19 pandemic. Other study options could include determining how the age of a plant or the quality of the soil and water around a plant impacts the phenological characteristics that it exhibits. A final future study option would be to create additional phenology trails in areas not affected by the urban heat island effect, such as Black Rock Forest, and trying to observe either the same or similar species on these new trails in order to provide more evidence for the impact of the urban heat island effect on New York City's plantlife.



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Figure 3: Phenophase calendar depicting the phenophases of all individuals of each species compared to typical trends.

February 13 2020

<u>May 3 2020</u>



Figure 4: Example of phenological changes observed in the three individuals of American Pokeweed in this study.

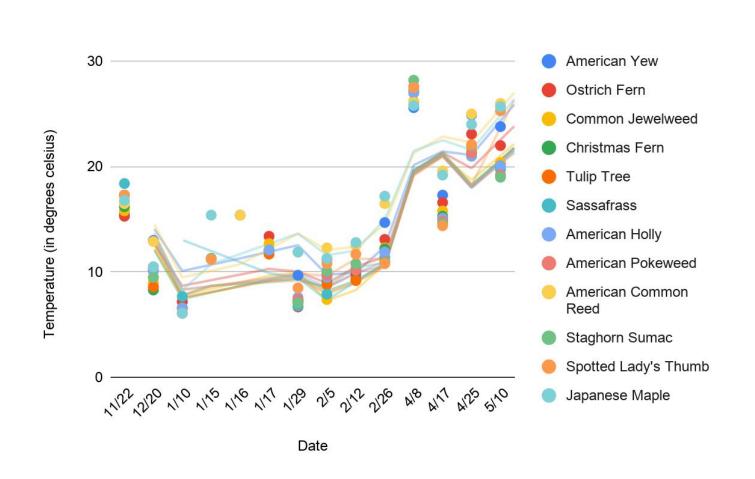


Figure 6: Temperature fluctuation graphs from October 2019 to May 2020 for Central Park study plant sites.

CSH Cold Spring Harbor Laboratory DNA LEARNING CENTER

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## Discussion

#### **Acknowledgements**

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