

- ## Investigating and Discovering a Correlation Between the Lichen Surrounding Bird Nests in the Garden City Bird Sanctuary



- Our samples of lichen such as *Parmelia sulcata* will be collected from the Garden City Bird Sanctuary. Originally a neglected and trash filled storm water basin (sump), the Garden City Bird Sanctuary is now a flourishing community nature preserve. The local green space has many habitats for organisms thrive in, with the use of native plants and trees to provide food and cover for the thriving ecosystems.

**Location:** Latitude: 40°43'55"N Longitude: 73°40'21"W.

- We will take photographs of the specimen, along with taking samples of the leaves surrounding the bird nests and placing them in jars or other containers. Using an application called iNaturalist, the photographs will be used to identify the type of lichen that surround or be in sparrow, hummingbird, and cardinal bird nests, without harming the actual habitat or ecosystem by taking a thin section of the lichen.
- We will be using nests that have been collected and put into display cases along with nesting boxes that are no longer in use, and also will be collecting samples outside of the breeding season to ensure that no harm will be inflicted upon the organisms there.
- Using the samples, we will use the Silica DNA method to isolate the DNA, and also enhance the DNA using PCR so it can be read. We will then use Gel Electrophoresis to compare and analyze the amplified DNA, where the procedure can be found at <https://dnabarcoding101.org/lab/>.

We have several goals regarding the experiment.

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- Agarose 2%
- 1 2 3 4 5 6 7 8 9 10

- Out of the samples we collect, the ones that will be successfully barcoded under Gel Electrophoresis will be analyzed to see how closely related the plant specimens are. We will observe the characteristics of the electrophoresis bands and notice their distance from each other, their similarity in frequencies, their intensity, and more. We will also observe the photos of the plants, and make a table of the similarities, differences, and characteristics of the organisms, so we will have a variety of information about the plants to compare and contrast with one another.
- We will also use DNA Subway to analyze the DNA, predicting and annotating the genes while also searching the genetic material for similarity with other sequences. DNA subway has 5 lines, 1) Annotate a genomic sequence, 2) Prospect Genomes using TARGet, 3) Determine Sequence Relationships, 4) Next Generation Sequencing, and 5) Metabarcoding Analysis. We will be using the Determine Sequencing Relationships line and using a fungal specific PCR primer to analyze the Internal Transcribed Spacer region of DNA. The ITS region is a highly variable sequence that is very important in distinguishing fungal species from one another. By putting the DNA into DNA Subway, we will make a model of our sequence and compare it to base pairs that scientists have provided in a database, Phytozome, to see the genetic diversities between the lichens.

- The DNA of archival nests is probably too degraded to obtain DNA from in traditional methods, so we do not know for sure if there is a correlation between the lichen and bird nests, or if they choose to locate in the same area.
- Based on the usual locations of cardinal, woodpecker, and other bird nests in trees along with the location of lichen on trees, it can be presumed that they may reside in similar environments.
- However due to the lack of clear evidence of if they do depend on each other, it can be presumed that they live independently of one another.
- The nest boxes actually have mice nests in them, so fresh nest samples cannot be obtained.
- With better DNA samples and more evidence, we could then make a more confident conclusion.

1) Oriole	1-1	1-2	1-3
2) Cardinal	2-1	2-2	2-3
3) Barn Swallow	3-1	3-2	3-3
4) Mourning Dove	4-1	4-2	4-3
5) Downy Woodpecker	5-1	5-2	5-3
6) Unknown	6-1	6-2	6-3

6) Unknown

[illegible]