Buggin' Out: Biodiversity of Long Island Mosquitos

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Abstract

Our team began this project with the intention of capturing and barcoding mosquitoes in order to determine the species of mosquito that inhabit Long Island. For our study, we set up a mosquito trap in a swampy area of CSHL using dry ice and premade bait as a lure. After a few days we opened the trap, examined the species, performed an extraction and electrophoresis, and sent them off for sequencing. We were pleased to find that the sequences, for the most part, were all of good quality. When actually running a BLAST for each of the sequences, however, we discovered that the insects we believed to be mosquitos were flies. While disappointed that we were not able to get an accurate representation of the mosquito population, we conclude with the statement that DNA barcoding is important to identifying a given organism. It's possible for one to realize something is entirely different from what they believed it to be.

Figure 1: Annika and Gavin
An image of us, and the trap we used to collect the specimens.

Background Info

Mosquitos are a vector of transmission for many diseases such as Malaria, West Nile Virus, Zika, and Dengue Fever. These diseases cause high fevers, possible birth defects, hemorrhages and in some cases, death. On Long Island specifically, the diseases West Nile Virus and Eastern Equine encephalitis are present. Our study involves the collection and barcoding of mosquitoes found at and in the surrounding area of Cold Spring Harbor Laboratory. With this process, we can figure out what species of mosquito inhabit the area, and subsequently, predict the other diseases that are present. The data we collect will also be useful to researchers who want to further examine the mosquito population. Such applications may include population statistics, invasive species research, and disease control and management.

Figure 2: The Asian Tiger Mosquito is a known invasive species that transmits viral infections.

Objectives/Hypothesis

As stated above, our research will serve to give a representation of the mosquito population. We hypothesize that we will not find any new invasive organisms, but rather only ones that have been found already. West Nile virus and Eastern Equine Encephalitis are the only common mosquito-borne illnesses on Long Island, and the Asian Tiger Mosquito (Aedes Albopictus) seems to be the only local (but still invasive) species that is a potential carrier.

Our data will serve to verify if this hypothesis holds true.

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Materials/Methods

I. Collection of Specimens

- We acquired 25 organisms through the use of a Biogents BG-Sentinel mosquito trap, using dry ice as bait. Our collection site was Cold Spring Harbor Laboratory. Due to the large similarity between some of the specimens, we only ran an extraction on 11 of the collected 25.

II. Extraction and Sequencing

- After cutting and placing pieces of each specimen in 1.5 ml test tubes, we used the standard DNA Barcoding 101 Silica protocol to extract and purify DNA from each sample. Next, through PCR, we copied and replicated the CO1 gene, verifying the integrity of each sample with electrophoresis. After the gel presented us with good results, we sent the samples off to be sequenced by GeneWiz.

III. BLAST/Barcoding

- In order to determine which species we had collected, we ran a nucleotide blast on each sequence we received from GeneWiz.

Figure 3: BG Sentinel Trap Placement
The location within the Cold Spring Harbor lab, in which our samples were collected.

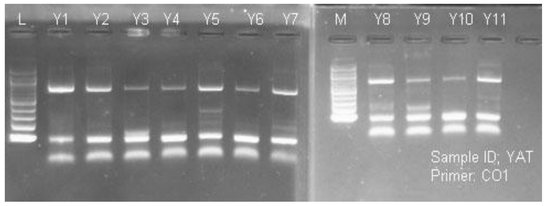

Figure 4: Gel Electrophoresis Results
The electrophoresis gel exhibits the quality of our samples. The proper application indicates that the PCR worked as planned--it cut and replicated a piece of DNA equal to the CO1 gene in length.

After receiving the sequences back, we ran a nucleotide BLAST and put our samples into the MUSCLE application in order to determine which mosquitoes we had collected.

Figure 5: MUSCLE results.
MUSCLE results show sequence alignment and mismatches.

Data

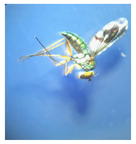

Figure 6: Sample 2

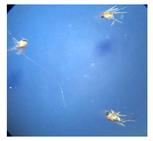

Figure 7: Sample 3

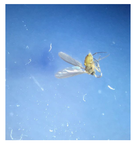

Figure 8: Sample 6

Figure 9: Sample 9

Figure 10: Sample 10

Figure 11: Sample 11

Sample Y01 is a match of 100% to the worm species *Cyathura polita*.

Sample Y02 is a match of 96.06% to the family Dolichopodidae in DNA Subway and BOLD.

Sample Y03 is a match of 100% to the Phoridae family in BOLD.

Samples Y05, Y07, and Y09 match genetically to each other. The closest match to these three samples in BOLD is to the family Sciardae at 100%.

Sample Y06 is a match of 96.64% to the family Phoridae in BOLD.

Sample Y11 is a match of 100% to the fly species *Megaselia arcticae*.

Sample Y10 is a match of 99.94% to Phoridae in BOLD.

Sample Y13 is a match of 96.10% to the order Diptera in BOLD.

In reality, it turns out that we had collected flies rather than mosquitos (the sequences generally were above a 95% match.) But the BLAST results showed only organisms of the Phoridae and Sciaridae *families*. While this is specific enough to tell us that we collected flies and gnats, the matches begin to lose confidence beyond this level. These organisms are not highly researched, and consequently there aren't sequences that match up with ours at the specificity of the species level.

CONCLUSIONS

As mentioned in the DATA section, electrophoresis of the samples showed great amplification of the CO1 gene, but our BLAST results showed that we did not obtain the mosquitos we thought we did. The sequences were almost complete matches to flies, mostly of the Phoridae and Sciaridae families.

Our team and advisors assume that the reason why we did not catch mosquitos is due to the onset of cold weather. We performed the collection on October 12, 2017: a date slightly after the mosquito population normally begins to die down due to the oncoming winter. Despite the mosquito population decreasing, the fly population remained. The flies were most likely attracted to the same bait, and flew into the trap.

As for the flies, the results were nonspecific and only told us (for the most part) only the families of the insects, so we obviously cannot make any conclusions regarding what species we have collected. On the bright side, a potential option for this situation is to target a different gene to barcode. Co1 is the most common taxonomic identification gene for animals, but it is possible there are sequences of a different gene for the species we collected--therefore allowing us to figure out what specific flies we captured.

While we are unable to provide data or a sound conclusion regarding the mosquito population, we hope our experiment serves to show the importance of DNA barcoding. The organisms that appeared as mosquitos had fooled us completely. They resembled known mosquitos--to the extent that we were confident in our identification of them--yet they were completely different insects. With DNA barcoding however, we realized our mistakes. We hope our audience learns from them.

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