Diversity at Upland Farm

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Abstract

In our project, we used DNA barcoding to identify mosquito species collected in the Upland Farm at Cold Spring Harbor Laboratory. It is very easy to misidentify species which have phenotype similarities and mosquitoes is one of the groups that are very difficult to identify taxonomically. Mosquitoes were collected and barcoded using COI to identify the species. Results shown that none of the sequence samples were mosquitoes. There were gnats and midges, which confirm the importance of DNA barcoding to identify specimenns

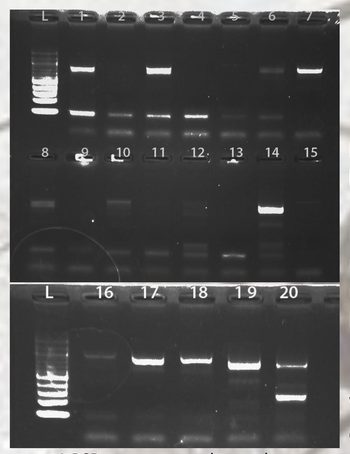
Introduction

Mosquitoes have been a rising problem in warmer areas, impacting populations by affecting health. Discovering which species are at the Uplands Farm (Figure 1) will help recognize the possible health implications the mosquitoes can have on the local population. It is also interesting to see if there was a change from the time it was a dairy farm to now as a research institution. Changes in temperatures will be recorded, and compared with the results of similar public projects.

Figure1. A) Upland farm map. **B)** Dr. Rob Martienssen and Novel laurate Barbara McClintock at the corn field.

Materials

Samples were collected at night with the BG-2 Sentinel Mosquito Trap and light traps.



A PCR assay was used to analyze

Methods

Extraction: We used the Silica protocol and then used washer buffer to purify our samples [5].

PCR amplification and gel electrophoresis: PCR primers were used for invertebrate COI gene using PCR. After using PCR, we used gel electrophoresis to confirm successful samples. Once we had our successful samples we used Genewiz for sequencing.

DNA Subway

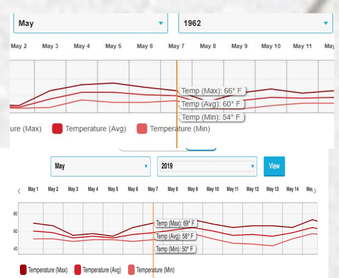
We then took the sequences to DNA to trim, align, and derive consensus sequencing. Further nucleotide blast was used to figure out the species.

Results

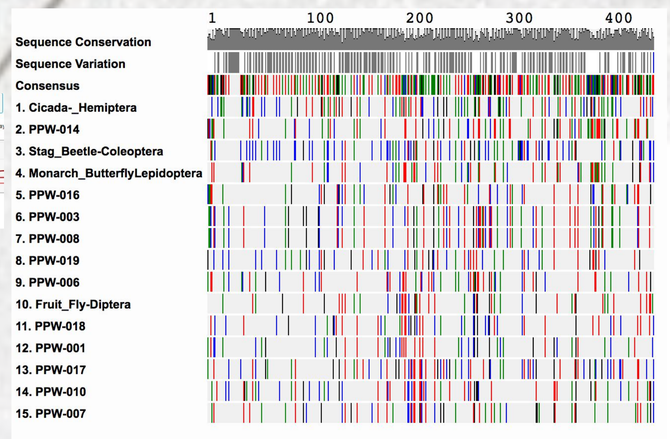
We analyzed 20 samples, We concluded that most of our samples have great differences based on the alignment viewer (Show a picture of the alignment). Luckily, 15 of our samples successfully amplified, but of the 15, 0 were mosquitos (Table 1). With this it became evident that all of the tested samples were different flies, with many unknowns about them.This was the recurring theme was very interesting because all of the samples looked incredibly alike mosquitos. The fact that all of our samples were flies, gives us confidence that there are a lack of vector for transmitting diseases.

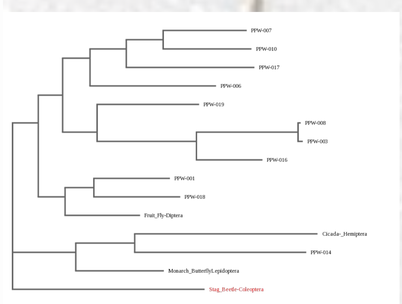
Temperature Analysis

We used the website, Weather Underground for very precise calculations[6]. The purpose of these visual representations is to show the environmental changes Upland Farms has underwent over the 57 year period. The maximum and minimum temperatures this year were both more extreme than those in 1962, but this year's average temperature was lower for May 7th.



This graph show the temperature from our collection day this year and in the year 1962. The visual representations have Maximum, Minimum, and Average Temperatures.





Discussion

Originally we predicted that our samples were in fact all mosquitos and taxonomically identified them as the Aedes species. After using the PCR Method for our extractions we came to conclusion that only 3 of our 20 samples were mosquitos yet none of the mosquitoes were amplified. Using the rest of the samples that were amplified, we then inserted each sequence into DNA Subway via BLASTN to reveal what each sequence actually was. The great diversity of species and lack of mosquitos gives a positive effect because the flies collected suggests that the are a lack of vectors for disease. Although our goal of understanding the biodiversity of mosquitoes was not reached, a greater understanding has been reached that is reassuring for natives.

Sequences	Taxonomic	Blast	Mismatches
PPW-001	Aedes	Oedalea Zetterstedti, Philipotabanus pterographicus	50, 49
PPW-003	Aedes	Sciaridae sp.	41
PPW-006	Aedes	Psychodidae sp., Psychoda alternata	0, 0
PPW-007	Aedes	Diptera sp., Chironomidae sp.	1, 9
PPW-008	Aedes	Sciaridae sp.	45
PPW-010	Aedes	Chironomus flaviplumus, Chironomus yoshimatsui	19, 20
PPW-014	Aedes	Cecidomyiidae sp., Diptera sp.	0, 4
PPW-016	Aedes	Bradysia impatiens	0
PPW-017	Aedes	Limnophyes sp.	0
PPW-018	Aedes	Dolichopodidae sp.	0
PPW-019	Aedes	Megaselia sp.	0

The table above includes each amplified sequence and its respective species along with their mismatches.

References

1. DNA Barcoding. (n.d.). Retrieved from https://www.sciencedirect.com/topics/medicine-and-dentistry/dna-barcoding
2. Kaufman, P.E, Harrington, L.C, Waldron, J.K., Rutz, D.A. 2005. The Importance of Agricultural Tire Habitats for Mosquitoes of Public Health Importance in New York State. Journal of the American Mosquito Control Association, 21(2):171-176.
3. The BG-Sentinel: Biogents' mosquito trap for researchers. (n.d.). Retrieved from https://www.bg-sentinel.com/
4. The Ultimate Guide to Mosquito Management on the Farm. (n.d.). Retrieved from https://www.mosquitomagnet.com/articles/guide-mosquito-management-farm
5. Uplands Farm. (n.d.). Retrieved from https://www.cshl.edu/research/plant-biology/uplands-farm/

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