Analyzing Diptera Biodiversity Using a C01 Gene in Farmingdale State University

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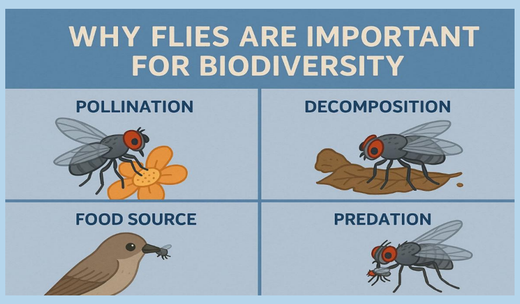
Abstract

Biodiversity is essential to stability in ecosystems and can be studied using DNA barcoding. The purpose of this study is to measure diptera biodiversity on Long Island using DNA barcoding techniques. To conduct this research, insects were photographed and documented after collection from a Malaise trap located in Farmingdale State University Teaching Gardens, and DNA was extracted using the Chelex protocol. The cytochrome oxidase 1 gene was amplified through the technique, polymerase chain reaction. Gel electrophoresis was ran to confirm the amplification. The resulting sequences were then analyzed in DNA Subway to identify and compare species using genetic sequences (bioinformatics). The Simpson's index was used to calculate biodiversity. The results indicated there was a high degree of biodiversity within the area. These results suggest that a stable, high-functioning ecosystem is present within the Farmingdale State Teaching Gardens Habitat. Future research could explore what specific roles each species occupy within the environment.

Introduction



Literature Review

Do Attractants Bias the Results of Malaise Trap Research?

Goal: The goal of this study is to prove whether or not different attractants could cause bias in the results of malaise trap research. It is hypothesized that when using denatured alcohol as an attractant, bias will occur in the results. This could lead to a misunderstanding of the diversity and ecology of insects.
Finding: The findings indicated that a much greater population of insects (625%) had been caught with the wet denatured ethanol traps when compared to the dry Vapona traps. The greatest increase of insects were those in the order Diptera. The data supports that there may be a bias based on the type of attractant used. Diptera were caught an average of 1,024% more using the denatured ethanol traps. The experiment revealed that attractants do bias the results of malaise trap research.

JEI. (2020). Do Attractants Bias the Results of Malaise Trap Research? | Journal of Emerging Investigators. Emerginginvestigators.org. https://emerginginvestigators.org/articles/19-010

Friend or Foe: Using DNA Barcoding to Identify Arthropods Found at Home

Goal: Aimed to determine if all types of arthropods found in houses across the world pose a harmful threat to humans.
Finding: Arthropods were collected from houses across a 12 month duration and DNA barcoding was used to identify them. After analyzing the data, it was found that none of the arthropods were on the pest list provided by the US government. Therefore, arthropods are not dangerous for humans and do not need to be immediately exterminated.

JEI. (2022). Friend or foe: Using DNA barcoding to identify arthropods found at home | Journal of Emerging Investigators. Emerginginvestigators.org. https://emerginginvestigators.org/articles/21-214

Research Question

Can the C01 gene in flies be used to measure and compare biodiversity in forest habitats?

Hypothesis

Ho- The CO1 gene is unable to be used to measure and compare fly biodiversity within forest habitats.
Ha- The C01 gene can be used to measure and compare fly biodiversity within forest habitats.

Methodology

Step 1 - Sample Collection

Figure 1: Map of GPS coordinates where samples were collected.

Step 2 - DNA Isolation

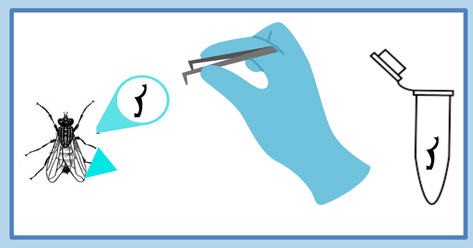


Figure 2: Removal of insect leg to extract DNA.

Step 3 - DNA Amplification

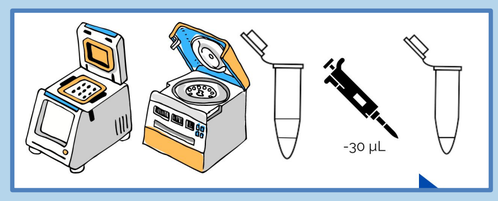


Figure 3: Specimen placed in a centrifuge to separate cellular debris from the DNA sample.

Step 4 - Gel Electrophoresis

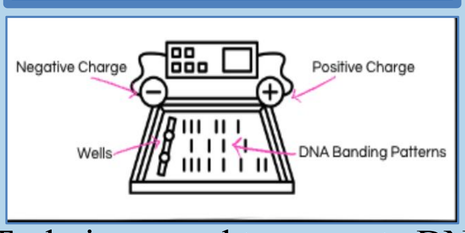


Figure 4: Technique used to separate DNA, RNA or proteins based on their size and charge.

Step 5- Bioinformatics

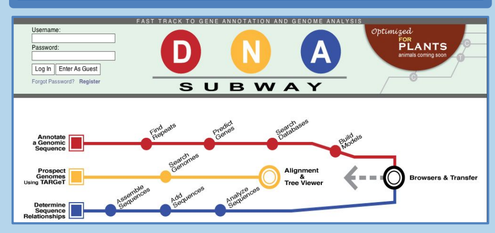


Figure 5: Website used to analyze and identify genes within a long DNA sequence.

Data

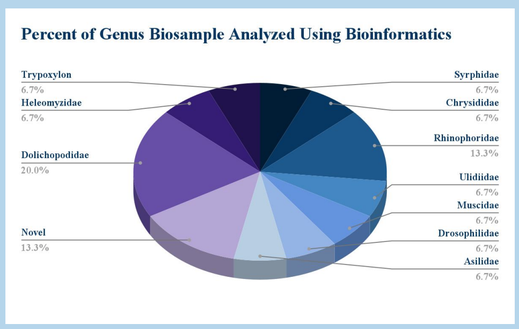
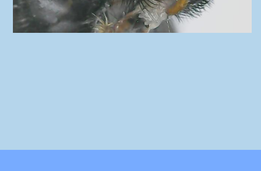


Figure 6: Pie chart depicting the Genus Biosample percents of diverse species.

Sample ID	Genus	Species	Family	ORDER	Forest Field or Ecotone
DCE-022	Ferdinandea	*buccata*	Syrphidae	Diptera	Forest
DCE-023			Chrysididae	Diptera	Forest
DCE-024	Melanophora	*roralis*	Rhinophoridae	Diptera	Forest
DCE-025			Ulidiidae	Diptera	Forest
DCE-026	Phaonia		Muscidae	Diptera	Forest
DCE-027	Melanophora	*roralis*	Rhinophoridae	Diptera	Forest
DCE-028	Drosophila	*robusta*	Drosophilidae	Diptera	Forest
DCE-029	Novel	Novel	Novel	Diptera	Forest
DCE-030	Neoitamus	*flavofemoratus*	Asilidae	Diptera	Forest
DCE-031	Gymnopternus	*opacus*	Dolichopodidae	Diptera	Forest
DCE-032	Suillia	*quinquepunctata*	Heleomyzidae	Diptera	Forest
DCE-033	Novel	Novel	Novel	Diptera	Forest
DCE-034	Sciapus	*spiniger*	Dolichopodidae	Diptera	Forest
DCE-035			Dolichopodidae	Diptera	Forest
DCH-026	Trypoxylon	*frigidum*		Hymenoptera	Forest






Results

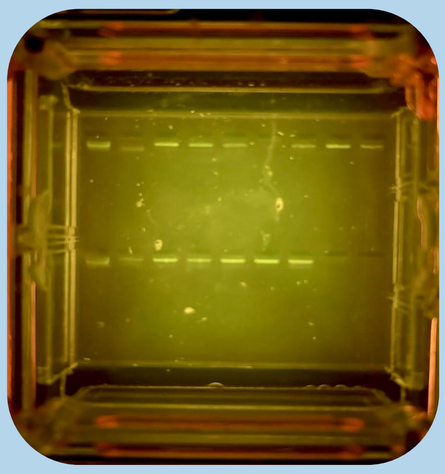


Figure 7: Image of a Gel Electrophoresis showing distinct DNA bands representing fragments that have moved through the gel based on their size and charge.

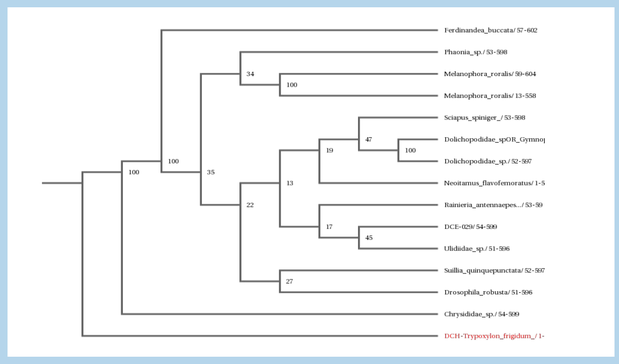


Figure 8: Phylogenetic Tree illustrating the evolutionary relationships among different insect species based on their genetic sequences.

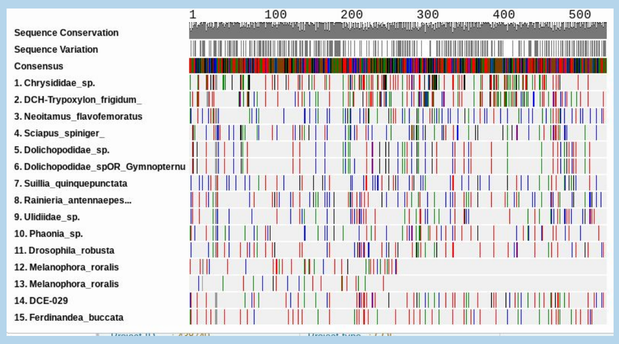


Figure 9: MUSCLE (Multiple Sequence Conservation Logic Engine) used to align DNA or protein sequences from different species. The variation of colors indicate where the sequences of 15 insect species are similar or different.

SIMPSON'S ANALYSIS

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

D=0.95

The biodiversity index was 0.9572, indicating a high degree of biodiversity.

Discussion

- Simpson's diversity index was calculated to determine biodiversity and a result of 0.9572 was obtained. This indicates a high level of biodiversity in the area, as it is extremely close to 1
- DCE-029 and Ulidiidae are genetically related 45% of the time according to the phylogenetic tree as shown in Figure 3
- Samples DCE-029 and DCE-033 were unable to be identified through the BOLD or BLAST system and therefore have been determined as novel
- According to the MUSCLE, species *Ferdinandea bucatta* and DCE-029 are most likely related genetically as they have similar alignment of DNA
- The largest percentage of Diptera was from the family Dolichopodidae and represented 20% of the samples