Differentiation of Suffolk Mosquito Samples by DNA Barcoding

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Abstract

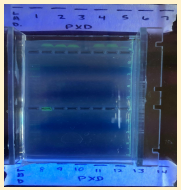
Mosquito samples collected from Connetquot State Park, *Aedes atlanticus* and *Aedes tormentor*, are from species not yet seen on Long Island, New York. These two species are difficult to distinguish morphologically from one another, for they have similar appearances. The goal for this project was to identify the unknown mosquito samples obtained from Connetquot State Park and confirm whether or not the mosquitos are either *Aedes atlanticus* or *Aedes tormentor*. In order to do so, we used a Chelex DNA isolation protocol, followed by an amplification protocol, which we then checked with a gel electrophoresis process. After the three times in which we tested our amplified samples through the agarose gel, none were successful; the DNA did not land around the estimated base bair amount for the mosquito samples. Unfortunately, our group concluded our experiment with no results.

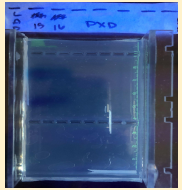
Introduction

Mosquito samples collected from Connetquot State Park are from species not yet seen on Long Island. These two species are difficult to distinguish morphologically from one another, for they have similar appearances. The mosquito samples collected are believed to be either the species *Aedes atlanticus* or *Aedes tormentor*, both of which are commonly found in the Southeast region of the United States. (Burkett-Cadena, 2013, pg. 81) Because these two mosquito species are not native to either the Northeastern region of the United States, there is a possibility that the mosquitos may be detrimental to the balance of the ecosystem.

Although mosquitos have positive influences on the environment, mosquitos are still considered as the "deadliest insect on Earth," for they transfer many life-threatening diseases from one organism to another. (Hadley, 2019) Some mosquito species can carry pathogens, especially those with the genus *Aedes*, *Culex*, or *Anopheles*. (CDC, 2020) These mosquitoes spread diseases through its bite; if a mosquito bites an infected host, the mosquito's next host will obtain the disease via the mosquito's bite. One of the most common mosquito-borne diseases in the United States is the West Nile virus, which can cause either mild flu-like symptoms or life threatening illnesses: meningitis, encephalitis, and meningoencephalitis. The West Nile virus is transmitted by mosquitoes exclusively with the genus *Culex*. (*West Nile Virus*) Another mosquito-borne disease is the Keystone virus. The Keystone virus was first reported in Keystone, Florida in several mammal populations, such as racoons, squirrels, and deer. (Morris, 2018) The virus is transmitted mainly by *Aedes atlanticus*, one of the possible species for the unknown mosquito samples obtained in the Long Island state park.

The Cytochrome oxidase 1 (CO1) gene is a popularly used gene in DNA barcoding for most eukaryotic organisms because it is a highly conservative gene region found in a species' mitochondria. This gene region can be used to identify individuals belonging to the same species or distinguish individuals belonging to a different species. (*The Ideal Barcoding Gene*, 2009). The CO1 gene, as well as other possible conservative genomic regions in eukaryotes, is going to be used for Chelex and/or Silica DNA extraction and isolation. From there, the isolated DNA will be sent for sequencing, and the sequence will then be used to genomically decipher if the mosquito samples obtained from Connetquot State Park are either *Aedes atlanticus* or *Aedes tormentor*, or possibly even both.





Results

Our results were inconclusive as shown on the images above. Since the amplification had not shown successful gel electrophoresis, the gels were not sent to the lab; therefore, there was no DNA barcode data for this experiment. The experiment for the mosquito DNA using chelex isolation, PCR, and gel electrophoresis was done three times all showing inconclusive results.

Discussion

The results from our group's experiments determined that our attempts at amplification were unsuccessful. This experiment included two DNA isolation procedures and three gel electrophoresis confirmations, with the first two gel electrophoresis confirmations resulting from the first DNA isolation and the third gel electrophoresis confirmation, which is shown above, resulting from the second DNA isolation. All of these gel electrophoresis confirmations showed no amplification of the DNA from the mosquito samples, thus rendering the DNA isolation for these samples inconclusive and unsuccessful. One possible explanation is that the primer used, the ant bead cocktail, is unsuccessful for mosquitoes or specifically this species of mosquito, and that the normal invertebrate COI primer may have worked better if this experiment were to be replicated. Similarly, the method for DNA extraction using the Chelex bead may have also led to the inconclusive data shown, and a more precise extraction such as the Silica isolation technique that is a less crude extraction of the DNA may have performed better. One other cause for the inconclusive data could have also been the capture of the mosquitoes. Since the mosquito samples were collected by and given to us by the Suffolk County Department of Health and not by our own capturing, the samples may have been tampered with, such as death of the samples before they were preserved in ethanol which could have left little to no cell material to extract DNA from.

If this experiment were to be conducted again, further investigation into the methods used would have to be undergone, such as the use of different primers and extraction methods as forementioned, since there were too many variables that could have gone wrong to pinpoint a single source for the inconclusivity of this experiment. Additionally, since the DNA Learning Center had originally advised for these specific techniques and protocols to be used, investigation into the reliability of the Ant Bead primer cocktail and/or the Chelex isolation technique may be a possible variable for the DNA Learning Center to monitor in other experiments with invertebrates that are not ants, as this problem could possibly be occurring for other groups as well.

Materials and Methods:

Chelex Isolation:

- 10% chelex solution (100 µL), 1 Sterile plastic pestle, Specimen tissue sample, Microcentrifuge tube lock, For sample storage: 95%+ EtOH [Ethanol] (1000 µL) or freezer, Permanent marker, Mug or container for boiling water, Aluminum foil , Optional: sterile razor blade, scissor, or pipette tip for tissue removal from specimen., Possible: Microcentrifuge tubes (1.5 mL); micropipette and tips (10-100 µL), microcentrifuge

Remove samples for 10+ minutes from ethanol to dry them out. Remove a tissue sample from the organism; place into a Chelex solution tube previously labeled with the sample-ID codes. Grind the tissue sample with a pestle; place and incubate the sample in the hot water bath at 95℃ for 10 minutes. Allow samples to cool for 10 minutes. Extract 30µL of the supernatant and transfer into a new 1.5mL centrifuge tube labeled PXD. Store sample at 4℃ or -20℃.

PCR Amplification:

- DNA extracted from organism(s), Container with cracked or crushed ice, Thermal cycler, Ready-To-Go-PCR Beads in 0.2-mL PCR tubes, COI primer cocktail (23µL per reaction)*, 2 Ready-To-Go PCR Beads in 0.2- or 0.5-mL PCR tubes, Appropriate primer/loading dye mix (50 µL; 23 µL per reaction), DNA from specimen(s) (from Part II)

Obtain the PCR tube with Ready-To-Go PCR Bead; label with the sample-ID number. Add 23µL of primer/loading dye mix; allow beads to dissolve for 1 minute. Place tubes on ice; add 2µL of previously obtained DNA. Run PCR amplification in the thermal cycler on the "Invertebrate (ant)" cycle.

Gel Electrophoresis:

- PCR products from PCR amplification, Container with ice, Micropipettes and tips (1-100 µL), Gel-casting tray and comb, Gel electrophoresis chamber and power supply, Microwave, UV or LED transilluminator, 2% agarose in 1X TBE (50 mL per), 1X TBE buffer (300mL per gel), Empty 1.5-mL microcentrifuge tube (10), SYBR Green DNA stain (25 µL), 100-bp ladder (10 µL per gel)*, PCR tube rack

Pour 2% agarose into the casting trays about ⅓ of the way up the combs, let the agarose solidify for 20 minutes. Remove the comb and place the solidified agarose gel into the electrophoresis chamber; fill the chamber with TBE buffer to cover the gels. Put 5µL of the PCR product and 2µL of SYBR green loading dye into a 1.5mL.

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