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The Effects of Mercury Exposure on the Cytochrome C Oxidase 1 Gene of Larval Dragonflies

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THE EFFECTS OF MERCURY EXPOSURE ON THE CYTOCHROME C OXIDASE 1 GENE OF LARVAL DRAGONFLIES

by

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ABSTRACT

Mercury is an environmental pollutant; its most toxic form is methylmercury. Once mercury is converted to methylmercury in a body of water it is able to bioaccumulate in organisms and biomagnify up the food chain. Mercury is able to cause DNA damage through the generation of free radicals and binding to sulfhydryl groups of cysteines in zinc finger DNA binding domains, inhibiting DNA repair machinery. In this study the potential mutagenic effects of mercury were investigated on larval dragonflies (Odonta: Anisoptera) collected from national parks across the United States. Since mercury is a known mutagen it was hypothesized that the COI gene from dragonfly larvae collected from sites where they are subjected to higher mercury exposure should have more mutations than the COI gene from dragonfly larvae collected from sites where they are subjected to lower mercury concentrations. The COI gene from each sample was first used to determine species of selected dragonflies through DNA barcoding then was analyzed for mutations in its nucleotide and amino acid sequences. Samples showing mutations in their amino acid sequences were modeled to determine if the mutation caused a change in the protein structure. Mutations were detected that did change protein structure, but at this point it is unknown if this structural change impacts protein function. The mutation rate was ultimately shown not to increase at sites with greater mercury exposure. Instead, the data suggests that genetic variation in dragonflies decreases at higher concentrations of mercury.

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INTRODUCTION

Mercury

Pollution

Mercury is an environmental toxicant with many natural and anthropogenic sources. The largest sources of mercury are oceans and other water bodies, but other natural sources include volcanic eruptions and wildfires.[1, 2, 3] Anthropogenic sources include gold mining and production, fossil fuel combustion, and cement production.[4, 5]

Mercury can be found in many different forms. Mercury emissions into the air take the form of elemental mercury or can be emitted as particulates. Through various oxidation-reduction reactions mercury can form soluble ionic or non-ionic organic or inorganic compounds.[1] The most prevalent form of mercury in the environment is methylmercury.[1, 6] Methylmercury present in aquatic environments is able to bioaccumulate in organisms and biomagnify up the food chain, entering cells by diffusing across cell membranes, due to being soluble in lipids.[7, 8, 9, 10] Once inside the cell, mercury induces a variety of genotoxic effects, such as mutations and chromosomal aberrations. The main ways that mercury causes DNA damage are generation of oxidative stress and interference with DNA repair enzymes.[7]

Genotoxic Effects

Oxidative stress is caused by the accumulation of reactive oxygen species (ROS).[7] Mercury allows for the production of ROS because it is easily oxidized by hydrogen peroxide, yielding the hydroxyl free radical.[11, 12, 13] While ROS can directly cause DNA damage, they also cause the generation of the modified nucleotide 8-oxo-deoxyguanosine

triphosphate (8-oxo-dGTP). This nucleotide can be incorporated in genomic or mitochondrial DNA, but the effects are more severe in the mitochondria because its polymerase, polymerase **γ**, suffers a decrease in fidelity in the presence of 8-oxo-dGTP.[14, 15, 16, 17] ROS can also damage cell membranes, particularly mitochondrial membranes, by inducing autocatalytic lipid peroxidation. In this process, the ROS oxidize a membrane lipid, turning it into an unstable fatty acid radical, prompting its degradation into a lipid hydroperoxide. This process makes the cellular membranes more permeable, and in the mitochondria this makes the electron transport chain less efficient, leading to the production of hydrogen peroxide.[18, 19, 20, 21]

Mercury can also cause an accumulation of DNA mutations due to interference with DNA repair machinery. This is due to the ability of mercury to bind to the sulfhydryl groups of the cysteines present in zinc finger DNA binding domains, which displaces the zinc ion and prevents DNA binding.[22, 23, 24] In a case study involving factory workers occupationally exposed to mercury vapor mercury exposure was shown to negatively impact base excision repair mechanisms more than nucleotide excision repair mechanisms.[22] Mercury can also bind the DNA directly, causing changes in secondary structure which could prohibit DNA machinery binding, or promote/inhibit translation.[7,25]

Damage Prevention

Cells produce glutathione (GSH), which acts as an antioxidant and functions to prevent DNA damage from mercury and the resulting ROS it generates. First, GSH contains a sulfhydryl group that allows it to bind to mercury. In this way GSH can sequester mercury compounds before they can be oxidized during the formation of ROS.[26] Second, GSH can neutralize any ROS made using mercury because it is easily

oxidized through the action of glutathione peroxidase.[26, 27, 28] Oxidized GSH can be recycled back to GSH by glutathione reductase in the presence of NADPH.[28]

GSH is produced by ϒ-glutamylcysteine synthatase and GSH synthase, but there is a feedback mechanism in place where GSH inhibits ϒ-glutamylcysteine synthatase function.[28, 29, 30, 31] This feedback mechanism is likely in place because if GSH is only partially oxidized, it becomes the glutathione thiol free radical and can increase the production of ROS.[26, 27, 32] GSH has been shown to be depleted in cells exposed to 1000 ppb of mercury, although DNA damage appears at a mercury concentration of only 50 ppb.[26]

DNA Barcoding

DNA barcoding allows for a species level identification for an organism using a short section of its genome. Identification is based on sequence homology of the section from the sample organism and sections from other organisms whose species was determined by taxonomists and vouchered in an authoritative repository. The Barcode of Life Database (BOLD) is one tool that can be used to make these comparisons (http://www.boldsystems.org).

After the initial set up and vouchering required by taxonomists, DNA barcoding is a more reliable way to identify many species for a few reasons. First, phenotypic variation within a population makes taxonomic identification of new or similar species difficult. Secondly, morphological keys used in identification are often specific for only one life stage and/or gender of a species. Lastly, because taxonomic identification requires specialized knowledge of morphological traits, taxonomists can often only identify the species of a small subset of organisms, whereas DNA barcoding is more

accessible.[33] For dragonflies, the adult lifestage is generally well known and described, but different species of larval dragonflies can be difficult to distinguish, in some cases requiring raising of a live larva until it matures.

DNA barcoding can be performed using ribosomal or mitochondrial DNA, however mitochondrial DNA is preferred because it has a higher rate of molecular evolution, allowing for discrimination between closely related species.[34] The mitochondrial cytochrome c oxidase I (COI) gene is currently the favored gene for DNA barcoding of insects like dragonflies because of the other mitochondrial protein-coding genes, COI exhibits the most rapid rate of molecular evolution of its third position nucleotides.[33, 35, 36] COI, along with the cytochrome c oxidase II (COII) gene, forms the final acceptor in the electron transport chain, catalyzing the reduction of oxygen to water.[37]

Dragonflies

The U.S. Geological Survey (USGS), the University of Maine, and the National Park Service (NPS) are currently involved in the collaborative Dragonfly Mercury Project. For this project, citizen scientists collect larval dragonflies from over 50 national parks, multiple sites per park, and the dragonfly larvae are analyzed for mercury content (Figure 1). In this way dragonflies act as biosentinels for the bioaccumulation of mercury in aquatic food systems.[38]

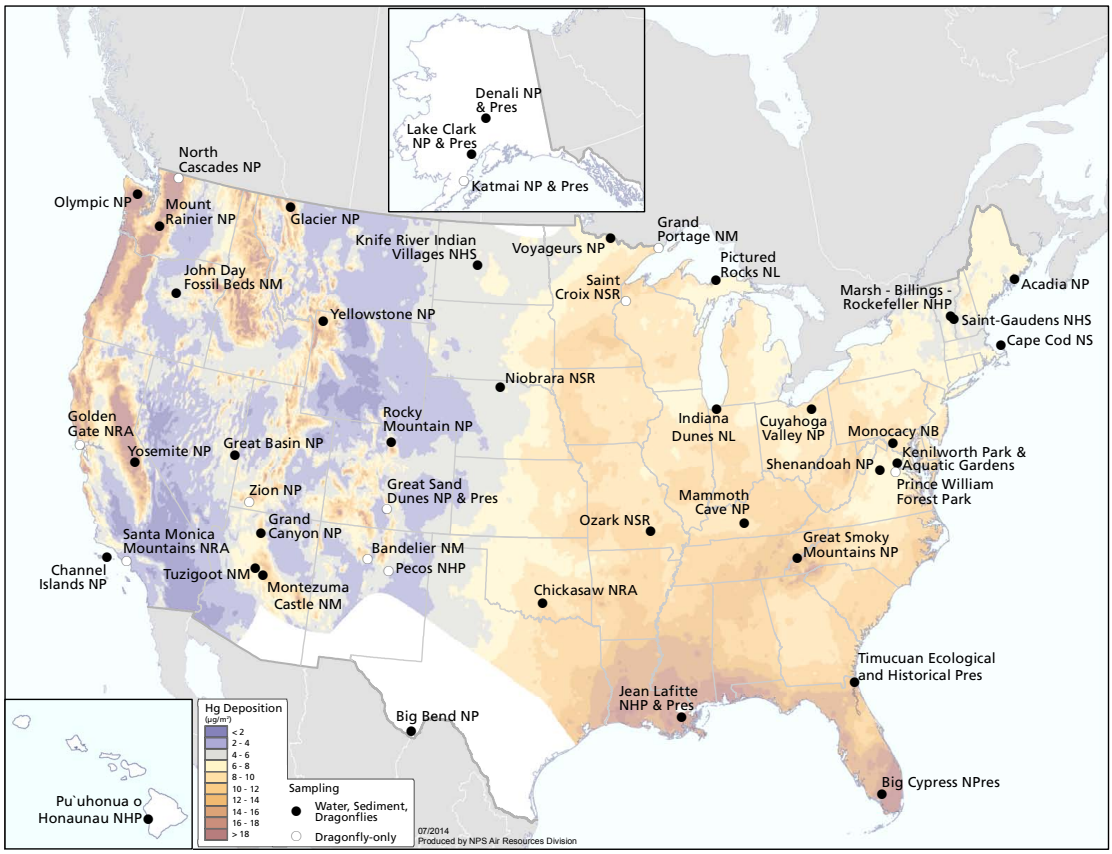

Figure 1: Parks that participated in the Dragonfly Mercury Project in 2014. Individual water bodies within the parks are separate sampling sites. Most samples in this thesis research were from parks in the eastern portion of the continental U.S.

For this project representative dragonflies from all sample sites were chosen for DNA barcode species identification. The COI sequences of select species were then analyzed for mutations and altered protein structure corresponding to level of mercury exposure. Since mercury is a known mutagen through its involvement in the generation of ROS and interference with DNA repair machinery, the COI gene from dragonfly larvae collected from sites where they are subjected to higher mercury exposure are expected to have more mutations than the COI gene from dragonfly larvae collected from sites where they are subjected to lower mercury concentrations.

MATERIALS AND METHODS

Sample Collection and Storage

Dragonflies collected from the field were individually bagged, shipped overnight on dry ice to the University of Maine, and stored at -20^{o}C. 215 samples representing all dragonfly families found at each sampling site were chosen to undergo DNA barcode species identification. The rest were sent to undergo mercury analysis.

Mercury Analysis

Wet weight of each sample was determined before freeze-drying and determination of dry weight. The University of Maine Sawyer Lab determined total mercury concentrations in dragonfly larvae using a Nippon Instruments Model MA-3000 Mercury Analyzer (Direct Hg by Thermal Combustion, US EPA method 7473). Mercury concentrations of samples found at the same sampling site were averaged to determine geometric mean concentration of mercury exposure in larvae at that site.

DNA Extraction

Tissue from three of each dragonfly's legs was used for the DNA extraction for each sample. The Qiagen DNeasy Blood and Tissue kit was used for DNA extractions. Leg tissue was combined with Buffer ATL and Proteinase K before being ground and incubated overnight at 55^{o}C. Samples were vortexed, combined with Buffer AL, vortexed again, and incubated at 55^{o}C for 20 minutes. After addition of ethanol, mixture volume was pipetted into a DNeasy Mini Spin Columns and centrifuged at 8000 rpm for 1 minute. Buffer AW1 was added and columns were again centrifuged at 8000 rpm for 1 minute. Buffer AW2 was added and columns were centrifuged at 14,000 rpm for 3

minutes. After placing column into a 1.5 mL microcentrifuge tube, Buffer AE was added, samples incubated at room temperature for 1 minute, and then were centrifuged at 8000 rpm for 1 minute to elute the DNA. Buffer AE was added again, samples were incubated at 55^{o}C for 5 minutes, and then were centrifuged at 8000 rpm for 1 minute to elute the remaining DNA. DNA was quantified using a nanodrop. Purified DNA was stored at -20^{o}C. See part B of Appendix for specific procedure.

Polymerase Chain Reaction (PCR)

m1COIintF and jgHCO2198 primers were used for amplification of extracted DNA. Amplified region was 313 bp in length.[34] Master mix contained the following component volumes per reaction: 12.5 µL Sigma RedTaq ReadyMix, 10.5 µL nuclease-free water, 0.5 µL m1COIintF, and 0.5 µL jgHCO2198. 24 µL of master-mix and 1 µL of DNA were used for each reaction. See part C of Appendix for thermal cycler conditions. PCR products were run on 1.5% agarose gels and photographed using a UV transilluminator before being stored at -20^{o}C.

DNA Sequencing

PCR products were cleaned using Affymetrix ExoSAP-IT, diluted to a concentration of 10 ng/ µL, and delivered to the University of Maine's DNA sequencing facility.

Sequence Analysis

Obtained nucleotide sequences were edited used 4Peaks software. Species was determined using the Barcode of Life Database (BOLD). Amino acid sequence generated using NCBI Blast. Nucleotide and amino acid sequence alignments were performed using Clustal Omega. Protein structure was determined using Phyre2.

RESULTS

COI Barcode Validity

Nucleotide and amino acid sequences from five representative species were aligned to test the validity of using COI gene sequences as barcodes for species identification (Figure 2). Nucleotide sequences have a number of variable loci, but due to the redundancy of the genetic code these are reduced in the amino acid sequences.

A

```
72      ---------------------------TCTGGCAGGTACAATTGCCCATGCTGGTTCCTC
73      TGGTACTGGTTGAACTGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
46      ---------------------------TTGGCTGGTGCAATTGCCCATGCAGGAGCATC
40      -GGTACTGGATGAACTGTTTATCCCCCTCTTGCAGGTGCAATTGCACATGCAGGAGCATC
82      -GGAACAGGATGAACAGTTTATCCCCCTTTAGCAGGAGCTATTGCTCATGCTGGTGCATC
                                     * ** **  * ***** ***** **  * **

72      TGTAGACCTAACAATTTTCTCTTTACACCTCGCAGGTGTATCATCAATTTTAGGAGCAAT
73      AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
46      AGTAGATCTAACTATTTTTTCTTTACATTTAGCTGGAGTATCTTCAATTCTTGGTGCTAT
40      AGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATCAATTCTAGGTGCTAT
82      AGTAGACTTAACTATTTTTTCCTTACACCTTGCAGGGGTATCTTCAATTCTAGGAGCTAT
         ** **  **** ** ** **  * **  * ** ** ** ** ****** * ** ** **

72      TAATTTTATTACCACTGTAATTAACATGAAATCACCAGGTATAAAATTAGACCAAATACC
73      TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
46      TAATTTTATTACTACAACAATTAATATAAAGTCACCAGGAATAAAGATAGATCAAATACC
40      TAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAAAATAGATCAAATACC
82      TAATTTTATTACAACAGTAATTAATATAAAATCACCAGGTATAAAAATAGATCAAATACC
        *** ******** **    ***** ** ** ******** ** **  **** ********

72      TCTATTTGTATGAGCGGTAGTAATTACAGCAGTGCTTTTACTATTATCTCTTCCTGTTTT
73      TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
46      TTTATTTGTATGAGCTGTTGTAATTACAGCTGTACTTTTATTATTATCATTACCTGTTTT
40      TTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTA-------------------
82      ACTATTTGTATGAGCGGTAGTAATTACTGCTGTTCTATTACTACTATCATTACCTGTTTT
          ********** ** ** ** ***** ** **  *  *

72      AGCAGGTGCTATTA-----
73      AGCAGGAGCCATTACCATA
46      GGCTGGTGCA---------
40      -------------------
82      AGCTGGTGCTATCACT---
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B

```
72      ---------LAGTIAHAGSSVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMP
82      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKMDQMP
73      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMP
40      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
46      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
                ***:*****:**************************.*********:****

72      ----------
82      ----------
73      LF--------
40      LFVWAVVITA
46      ----------
```

Figure 2: Multispecies alignment. Sample species are as follows: 72 – *Macromia illinoiensis*, 73 – *Gomphus Exilis*, 46 – *Aeshna umbrosa*, 40 – *Basiaeschna janata*, 82 – *Celithemis elisa*. A. Nucleotide sequence alignment. B. Amino acid sequence alignment.

Nucleotide Sequence Alignments

Nucleotide sequence alignments were performed on species found at more than one site and with total mercury concentrations ranging from <100 ppb dw (parts per billion, dry weight) to >200 ppb dw to detect possible mutations. Five species were examined (Figures 3 – 7). Each species was shown to have one sample with a mutation.

A

```
65      --------------------------TCTTGCAGGAGCAATTGCACATGCAGGAGCATC
153     -GGTACAGGATGAACTGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
137     -GGTACAGGATGAACTGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
73      TGGTACTGGTTGAACTGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
173     TGGTACAGGTTGAACTGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
208     --------------------------TCTTGCAGGAGCAATTGCACATGCAGGAGCATC
191     ------------------------CCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
178     ---------------------------CTTGCAGGAGCAATTGCACATGCAGGAGCATC
84      -------------------------------CAGGAGCAATTGCACATGCAGGAGCATC
81      TGGAACAGGATGAACAGTTTATCCCCCTCTTGCAGGAGCAATTGCACATGCAGGAGCATC
                                        ****************************

65      AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTATAGGAGCAAT
153     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
137     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
73      AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
173     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
208     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
191     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
178     AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
84      AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
81      AGTTGATTTAACTATCTTCTCTCTTCATCTTGCAGGAGTTTCATCAATTCTAGGAGCAAT
        ********************************************** **********

65      TAACTTTATTACTACAAATATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
153     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
137     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
73      TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
173     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
208     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
191     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
178     TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
84      TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
81      TAACTTTATTACTACAACTATTAATATAAAGTCACCAGGAATGAAATTAGACCAAATACC
        ********** ****************************************

65      TCTATTGGTATGGGCTGTAATTATTACTGCAGTTTT----------------------
153     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTAC-------------------
137     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTAC-------------------
73      TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
173     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTACA
208     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
191     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
178     TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
84      TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTT----------------------
81      TCTATTTGTATGGGCTGTAGTTATTACTGCAGTTTTACTTTTATTATCTTTACCAGTATT
        ****** ************ ***************
```

B

Sample #	Park Code	Site	[Hg] (ppb)	% Sequence Similarity
65	VOYA	Peary Lake	76	98.2
153	ACAD	Long Pond	131	100
137	ACAD	Jordan Pond	145	100
73	CACO	Herring Pond	79	100
173	ACAD	Hodgdon Pond	165	100
208	ACAD	Lakewood	130	100
191	ACAD	Aunt Betty's	234	100
178	ACAD	Eagle Lake	209	100
84	CACO	Great Pond	186	100
81	CACO	Duck Pond	171	100

Figure 3: A. *Gomphus exilis* nucleotide sequence alignments. B. Quantification of part A. Park codes are as follows: VOYA – Voyageurs National Park, CACO – Cape Cod National Seashore, ACAD – Acadia National Park

A

```
59        ACATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGATGGTGTATCATC
40        ACATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
50        ACATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
64        ACATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
88        ACATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
205       -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
196       -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
149       -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
135       -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
55        -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
54        -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
41        -CATGCAGGAGCATCAGTAGATTTAACTATTTTTTCTTTACACTTGGCTGGTGTATCATC
           ******************************************** ************

59        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
40        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
50        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
64        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
88        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
205       AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
196       AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
149       AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
135       AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
55        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
54        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
41        AATTCTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAATCACCAGGAATGAA
          ************************************************************

59        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
40        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTA-----
50        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
64        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
88        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTAGT
205       AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
196       AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
149       AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTG------------
135       AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATT---
55        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
54        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
41        AATAGATCAAATACCTTTATTTGTATGAGCAGTTGTAATTACAGCTGTACTCTTATTACT
          *********************************************
```

B

Sample #	Park Code	Site	[Hg] (ppb)	% Sequence Similarity
59	VOYA	Peary Lake	76	99.5
40	PIRO	Beaver Lake	142	100
50	PIRO	Grand Sable Lake	149	100
64	VOYA	Ryan Lake	153	100
88	OZAR	Coppermine Creek	136	100
205	ACAD	Aunt Betty's	234	100
196	ACAD	Jordan Pond	145	100
149	ACAD	Long Pond	131	100
135	ACAD	Jordan Pond	145	100
55	SACN	Earl Landing	135	100
54	SACN	Earl Landing	135	100
41	PIRO	Grand Sable Lake	149	100

Figure 4: A. *Basiaeschna janata* nucleotide sequence alignments. B. Quantification of part A. Park codes are as follows: PIRO – Pictured Rocks National Lakeshore, SACN – Saint Croix National Scenic Riverway, VOYA – Voyageurs National Park, OZAR – Ozark National Scenic Riverway, ACAD – Acadia National Park.

A

```
186       ------------------------CCTCTGGCAGGTACAATTGCCCATGCTGGTTCCTCT
72        ------------------------TCTGGCAGGTACAATTGCCCATGCTGGTTCCTCT
134       GGTACTGGTTGAACAGTTTATCCCCCTCTGGCGGGTACAATTGCCCATGCTGGTTCCTCT
85        GGAACAGGATGAACAGTTTATCCCCCTCTGGCAGGTACAATTGCCCATGCTGGTTCCTCT
                                  ****** **************************

186       GTAGACCTAACAATTTTCTCTTTACACCTCGCAGGTGTATCATCAATTTTAGGAGCAATT
72        GTAGACCTAACAATTTTCTCTTTACACCTCGCAGGTGTATCATCAATTTTAGGAGCAATT
134       GTAGACCTAACAATTTTCTCTTTACACCTCGCAGGTGTATCATCAATTTTAGGAGCAATT
85        GTAGACCTAACAATTTTCTCTTTACACCTCGCAGGTGTATCATCAATTTTAGGAGCAATT
          ************************************************************

186       AATTTTATTACCACTGTAATTAACATGAAATCACCAGGTATAAAATTAGACCAAATACCT
72        AATTTTATTACCACTGTAATTAACATGAAATCACCAGGTATAAAATTAGACCAAATACCT
134       AATTTTATTACCACTGTAATTAACATGAAATCACCAGGTATAAAATTAGACCAAATACCT
85        AATTTTATTACCACTGTAATTAACATGAAATCACCAGGTATAAAATTAGACCAAATACCT
          ************************************************************

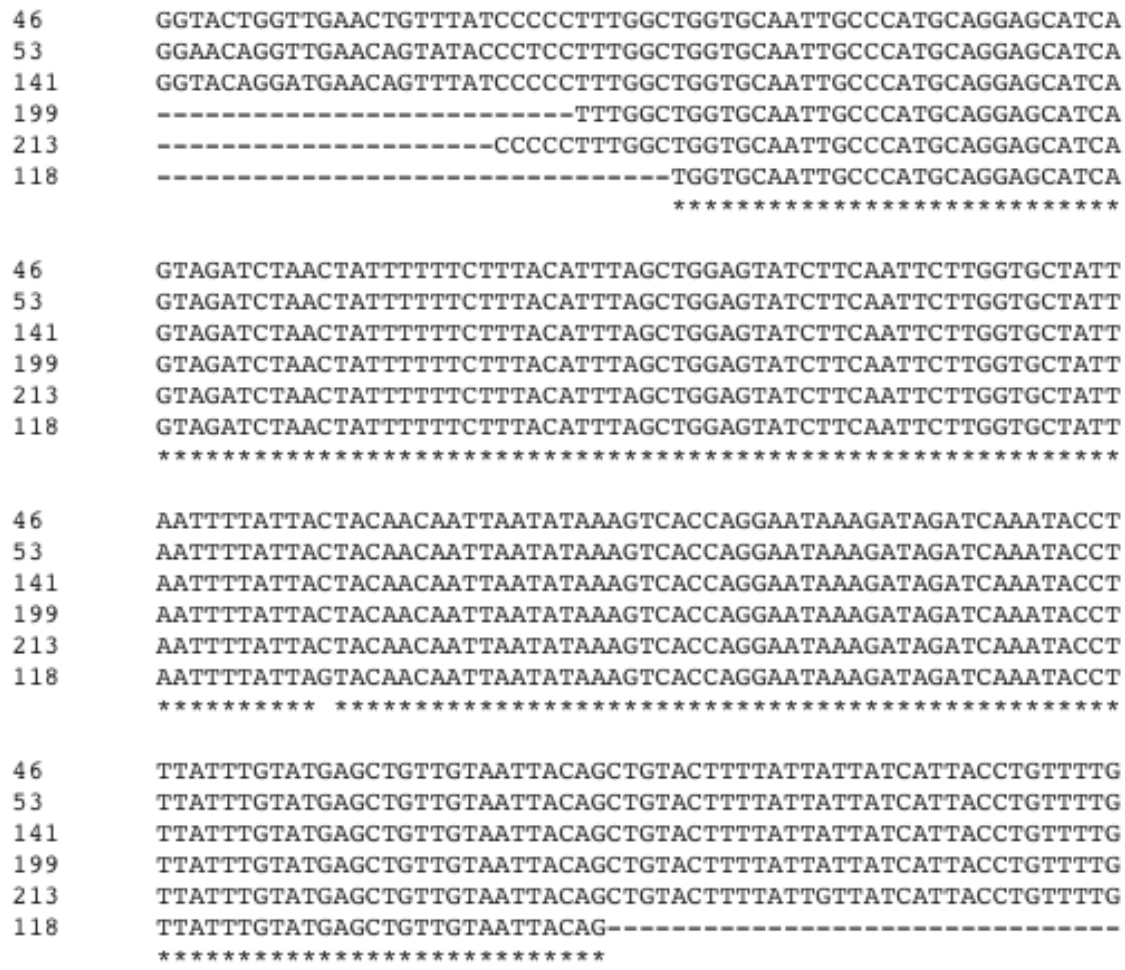
186       CTATTTGTATGAGCGGTAGTAATTACAGCAGTGCTTTTACTATTATCTCTTCCTGTTTTA
72        CTATTTGTATGAGCGGTAGTAATTACAGCAGTGCTTTTACTATTATCTCTTCCTGTTTTA
134       CTATTTGTATGAGCGGTAGTAATTACAGCAGTGCTTTTA--------------------
85        CTATTTGTATGAGCGGTAGTAATTACAGCAGTGCTTTTACTATTATGGTCTTCCTGTTTT
          ************************************
```

B

Sample #	Park Code	Site	[Hg] (ppb)	% Sequence Similarity
186	ACAD	Eagle Lake	209	100
72	CACO	Gull Pond	70	100
134	CACO	Duck Pond	171	99.5
85	CACO	Great Pond	186	100

Figure 5: A. *Macromia illinoiensis* nucleotide sequence alignments. B. Quantification of part A. Park codes are as follows: CACO – Cape Cod National Seashore, ACAD – Acadia National Park.

A



B

Sample #	Park Code	Site	[Hg] (ppb)	% Sequence Similarity
46	PIRO	Legion Lake	173	100
53	SACN	Phipps Landing	96	100
141	ACAD	Eagle Lake	209	100
199	ACAD	Lakewood	130	100
213	ACAD	Schoodic	732	100
118	GRPO	Poplar Creek	140	99.5

Figure 6: A. *Aeshna umbrosa* nucleotide sequence alignments. B. Quantification of part A. Park codes are as follows: PIRO – Pictured Rocks National Lakeshore, SACN – Saint Croix National Scenic Riverway, GRPO – Grand Portage National Monument, ACAD – Acadia National Park

A

```
140     AGGAACTGGATGAACTGTTTATCCCCCTTTAGCAGGAGCTATTGCTCATGCTGGTGCATC
82      -GGAACAGGATGAACAGTTTATCCCCCTTTAGCAGGAGCTATTGCTCATGCTGGTGCATC
204     ---------------------------TTTAGCAGGAGCTATTGCTCATGCTGGTGCATC
                                   ********************************

140     AGTAGACTTAACTATTTTTTCCTTACACCTTGCAGGGGTATCTTCAATTCTAGGAGCTAT
82      AGTAGACTTAACTATTTTTTCCTTACACCTTGCAGGGGTATCTTCAATTCTAGGAGCTAT
204     AGTAGACTTAACTATTTTTTCCTTACACCTTGCAGGGGTATCTTCAATTCTAGGAGCTAT
        ************************************************************

140     TAATTTTATTACAACAGTAATTAATATAAAATCACCAGGTATAAAAATAGATCAAATACC
82      TAATTTTATTACAACAGTAATTAATATAAAATCACCAGGTATAAAAATAGATCAAATACC
204     TAATTTTATTACAACAGTAATTAATATAAAATCACCAGGTATAAAAATAGATCAGATACC
        ************************************************** *****

140     ACTATTTGTATGAGCGGTAGTAATTACTGCTGTTCTA----------------------
82      ACTATTTGTATGAGCGGTAGTAATTACTGCTGTTCTATTACTACTATCATTACCTGTTTT
204     ACTATTTGTATGAGCGGTAGTAATTACTGCTGTT-------------------------
        ********************************
```

B

Sample #	Park Code	Site	[Hg] (ppb)	% Sequence Similarity
140	ACAD	Jordan Pond	145	100
82	CACO	Gull Pond	70	100
204	ACAD	Eagle Lake	209	99.5

Figure 7: A. *Celithemis elisa* nucleotide sequence alignments. B. Quantification of part A. Park codes are as follows: CACO – Cape Cod National Seashore, ACAD – Acadia National Park

Amino Acid Sequence Alignments

Amino acid sequence alignments were performed on samples shown by the nucleotide sequence alignments to be mutated (Figure 8). Samples were compared to one non-mutated representative sample of the same species. Two samples were shown to possess nucleotide mutations that translated into amino acid mutations.

A

```
65      LAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLF
73      LAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLF
        ***************************************************:******
        1                                                       53
```

B

```
59      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
40      GTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
        ************************************************************
        1                                                           60
```

C

```
72        LAGTIAHAGSSVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMP
134       LAGTIAHAGSSVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMP
          ***************************************************
          1                                                 51
```

D

```
118       GAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
46        GAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMP
          ************************************************
          1                                               49
```

E

```
82      LAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKMDQMP
204     LAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMP
        ***************************************************:****
        1                                                   51
```

F

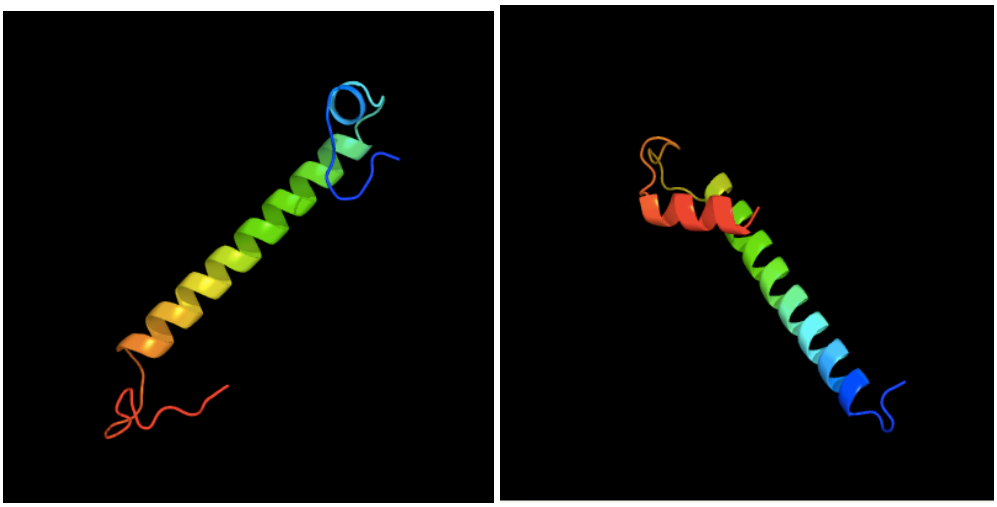
Sample #	Species	Park Code	Site	[Hg] (ppb)	Amino Acid Changes
65	*G. exilis*	VOYA	Peary Lake	76	L47M
59	*B. janata*	VOYA	Peary Lake	76	None
134	*M. illinoiensis*	CACO	Duck Pond	171	None
118	*A. umbrosa*	GRPO	Poplar Creek	140	None
204	*C. elisa*	ACAD	Eagle Lake	209	M47L

Figure 8: A – E Amino acid sequence alignments for *G. exilis*, *B. janata*, *M. illinoiensis*, *A. umbrosa*, and *C. elisa*. Numbers in red represent amino acid position. F. Quantification of mutated samples. Park codes are as follows: VOYA – Voyageurs National Park, ACAD – Acadia National Park, CACO – Cape Cod National Seashore, GRPO – Grand Portage National Monument

Protein Structure Models

Models of protein structure for the samples with mutated amino acid sequences were compared to models generated from the non-mutated amino acid sequences of samples of the same species (Figure 9). Both non-mutated sequences generated a structure consisting of a single alpha helix, while both mutated sequences generated a structure consisting of a double alpha helix.

A



B

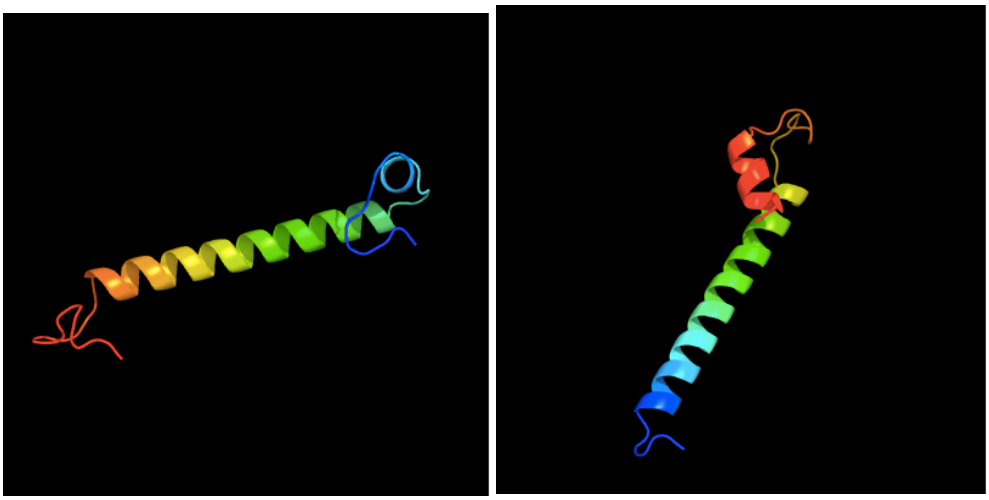


Figure 9: Protein Structure Models. Samples are rainbow colored starting at the N-terminus, in red. A. *G. exilis* samples 73 (non-mutated, left) and 65 (mutated, right). B. *C. elisa* samples 82 (non-mutated, left) and 204 (mutated, right).

DISCUSSION AND CONCLUSIONS

The cytochrome c oxidase I (COI) gene is widely used for DNA barcoding and Figure 2 illustrates why this gene is so effective for species identification. In parts A and B, the nucleotide and amino acid sequences of five different dragonfly species are aligned. Part A shows that while the genes maintain enough sequence identity to be aligned, there are many variations in the nucleotide sequence. Despite these variations, the redundancy of the genetic code ensures nearly identical amino acid sequences, shown in part B. This is expected because the COI protein structure would need to be conserved for it to function properly during oxidative phosphorylation.

Despite COI being well suited for DNA barcoding, only 41% of samples were successfully identified to the species level due to poor sequencing. It is suspected that the PCR primers may not have been optimal for all genus or species. Primers used were developed relatively recently as an alternative for the traditional primers to yield a 313 bp PCR fragment rather than a 658 bp PCR fragment to enable easier sequencing. The 313 bp fragment is the last 313 bp of the 658 bp fragment.[34] It is advisable in the future to conduct a comparative study of the efficiency of the two primer sets as the original comparison was evaluated in fish, so perhaps the primers are not well suited for dragonflies.

Many studies have documented that mercury is able to bioaccumulate in dragonflies, however the effects of mercury on dragonflies has not been well investigated.[39, 40, 41] Mercury has been shown in other organisms to act as a mutagen. In a study using mouse embryonic fibroblasts transfected with the *lacZ* reporter gene, mercury

exposure was shown to cause DNA mutations at a concentration of 50 ppb. Mutation frequencies at 100 ppb and 150 ppb were not significantly different from each other, but were higher than at 50 ppb. Mercury concentrations higher than 150 ppb were not tested[26]. The potential of mercury to cause DNA damage at these concentrations in other organisms is significant because the State of Maine fish consumption advisory concentration of mercury is 200 ppb.[42] While there are no published thresholds to indicate concentrations of mercury that would have species, population, or higher trophic level consumption implications for dragonflies, because they are known vectors for mercury biomagnification the effects of mercury on these organisms is worth investigating.

To attempt to find any mutations in the COI gene corresponding to mercury exposure, the nucleotide sequences were aligned from dragonfly species found to be living in habitats associated with relatively high and relatively low dragonfly larvae mercury concentrations (at least one habitat <100 ppb dw and one >200 ppb dw). Five species fit this description: *Gomphus exilis, Basiaeschna janata, Macromia illinoiensis, Aeshna umbrosa*, and *Celithemis elisa*. It was hypothesized that samples found at sites that would subject them to a relatively higher concentration of mercury would have more mutations than samples found at sites that would subject them to a relatively lower concentration of mercury. As shown in Figures 3 – 7, each species had one representative member with at least one mutation. All mutations detected were point mutations. This is consistent with the findings of a human population study that studied the DNA repair efficiency of lymphocytes taken from factory workers occupationally exposed to

mercury. The study concluded that mercury exposure impairs base excision repair mechanisms.[22]

With the exception of *C. elisa* sample 204, the rest of the mutated samples were from sites that contained dragonflies with less than 200 ppb of mercury, on average. These findings agree with the previously mentioned mouse model study in the sense that DNA mutations did occur at in dragonflies at the same mercury concentrations that they occurred in mice, but mutation frequency did not show a trend of increasing as mercury concentration increased, which did not support the hypothesis. The chi-squared statistic for this data was calculated to be 2.263 with a p-value of 0.323, which is not significant at $p < 0.05$. While the result of most sequence variation occurring at mercury concentrations less than 200 ppb was unexpected, it suggests that genetic variation within a species is reduced in areas with a higher mercury concentration. As there was only one sample site tested with a mercury concentration significantly above 200 ppb, this cannot be definitively concluded.

Point mutations in the coding region of a nucleotide sequences are the most dangerous if they lead to changes in the amino acid sequence, which could change in the protein structure to reduce or inhibit function. To test for this, the amino acid sequences of the samples with mutated nucleotide sequences were aligned with the amino acid sequences of samples of the same species whose nucleotide sequences weren't mutated. For three samples, the mutation(s) present in the nucleotide sequence caused no change in the amino acid sequence (Figure 8), but for two samples these mutations led to mutations in the amino acid sequence. *G. exilis* sample 65 underwent a Leucine to Methionine mutation, and *C. elisa* sample 204 underwent a Methionine to Leucine mutation. Both

mutations occurred at the same locus. Looking back at the nucleotide sequences for these two samples, they both underwent mutations that led to the incorporation of a guanine. It is possible this guanine is 8-oxo-dGTP, in which case this mutation would be the result of oxidative damage.[14, 15, 16]

To determine if these amino acid mutations altered the protein structure of COI, models of the structures were generated, shown in Figure 9. Both of the non-mutated control samples had a secondary structure consisting of a single alpha-helix. Both mutated samples had altered structures consisting of a large alpha-helix accompanied by a smaller alpha-helix. It is interesting that despite these mutations occurring at the same locus and being essentially the reverse of each other, that they both caused the same change in secondary structure. It is possible that this change in structure could impact COI function.

These results show that while more mutations were not found in samples from sites with relatively higher concentrations of mercury, some mutations that did occur caused alterations to the amino acid sequence and protein structure of COI. Despite mercury being a known mutagen, because these dragonflies were collected from the environment we cannot be certain that mercury exposure was the cause of the detected mutations. They could have been exposed to another, un-tested mutagen before being collected. It is also possible that these mutations are just contributing to the natural variation present between individuals and simply happened over time.

Future experiments need to be performed to draw more definitive conclusions from these results. Analysis should be performed on COI mutants with altered protein structure to determine if they are still functional and in what capacity. To determine if

mercury is actually reducing genetic variation at concentrations over 200 ppb, a greater range of concentrations need to be analyzed than those examined in this study. If sampling sites at this range are not available, this could be done using artificial exposure of dragonflies to mercury in a lab. Artificial exposure would also help to determine if the mutations shown are actually being caused by mercury because researchers could limit exposure to other pollutants.

As this was the first study of its kind (to our knowledge), dragonfly leg tissue was used for mutation analysis so taxonomic identification of samples would still be possible in the event DNA barcoding failed. In the future results may be improved by utilizing abdomen tissue as it has been shown to be the body region where mercury accumulates the most in dragonflies.[39] However, it should be noted that because mitochondria are maternally inherited, and COI is a mitochondrial gene, detected mutation may have been maternally inherited, and in this case would not be specific to a particular body region.

This study was conducted with a very small sample size, which also impacts the ability to draw strong conclusions. The samples are randomly collected from the field and species is identified later, so ensuring the collection of a certain number of species is impossible. However only one year was examined for this study. Examining multiple years' worth of data could help to increase the sample size and thus strengthen the conclusions.

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APPENDIX

Standard Operating Procedure for DNA Barcoding of Larval Dragonflies

Megan Little

University of Maine

Karen James, Michael Marion - MDI Biological Laboratory

Hamish Grieg, Sarah J. Nelson – University of Maine

A contribution to the Dragonfly Mercury Project

A. Preparing the Tissue Samples

Materials and Equipment:

- Preserved[1] dragonflies
- Glass petri dish
- Kimwipes®
- Forceps
- Scissors
- 1.5 mL microcentrifuge tubes
- Flame source (Bunsen burner, lighter, etc.)

Procedure:

1. Sterilize all instruments and the glass petri dish by passing them over a flame before beginning the dissections.
2. Working with one dragonfly at a time, cut all the legs off one side of the dragonfly using the scissors.
3. Place all legs into one microcentrifuge tube labeled with the sample ID and date. If not moving directly to part C, freeze sample in -20°C freezer.
4. Sterilize all instruments used in the dissection, including the glass dish. Replace the Kimwipe® on the work surface.
5. Repeat procedure for all dragonflies being analyzed.

B. DNA Extraction

Materials and Equipment:

- Qiagen DNeasy Blood and Tissue Kit
- 1.5 mL microcentrifuge tubes
- Heat block or oven
- Microcentrifuge
- Vortex
- Pipettes and sterile, disposable pipette tips (ideally filter tips)
- Disposable plastic pestles

Procedure:

1. Add 180 µL of Buffer ATL and 20 µL Proteinase K to each sample and vortex for 5 seconds. Incubate overnight at 55°C.
2. Vortex each sample for 15 seconds, add 200 µL Buffer AL, then grind tissue with a new plastic pestle. Vortex again and incubate at 55°C for 20 minutes.
3. Add 200 µL of ethanol to each sample and vortex.
4. Working with each sample separately, pipette the entire mixture volume into a DNeasy Mini Spin Column placed in a 2 mL collection tube. Centrifuge at 8000 rpm for 1 minute in a microcentrifuge.
5. Discard the flow-through and replace the collection tube. Pipette 500 µL Buffer AW1 into the column and centrifuge at 8000 rpm for 1 minute.

[1] Dragonflies may be preserved by either freezing or submersion in 95% ethanol.

6. Discard the flow-through and replace the collection tube. Pipette 500 µL Buffer AW2 into the column and centrifuge at 14,000 rpm for 3 minutes.
7. Discard the flow-through and discard the collection tube, placing the column into a 1.5 mL microcentrifuge tube.
8. Pipette 100 µL Buffer AE into the column and incubate at room temperature for 1 minute. Centrifuge at 8000 rpm for 1 minute to elute the DNA.
9. Pipette 50 µL of Buffer AE into the column and incubate at 55^{o}C for 5 minutes. Centrifuge at 8000 rpm for 1 minute to elute the remaining DNA.
10. Discard the column and quantify the DNA using the nanodrop.
11. Store the purified DNA in a -20^{o}C freezer if not moving directly to part D.

C. PCR

Materials and Equipment:

- m1COIintF and jgHCO2198 primers
- Sigma RedTaq ReadyMix
- Purified DNA
- Microcentrifuge or mini-microcentrifuge
- Pipettes and sterile, disposable pipette tips (ideally filter tips)
- Thermocycler

Procedure:

1. Use http://www.idtdna.com/Calc/resuspension to calculate how much nuclease-free water is needed to resuspend your new primers to form a 100 µM stock solution. Make an aliquot of 10 µM working solution.
2. Make your master-mix in a 1.5 mL microcentrifuge tube using the following component volumes per reaction, including a negative control and 2 extra reactions: 12.5 µL Sigma RedTaq ReadyMix, 10.5 µL nuclease-free water, 0.5 µL forward primer, and 0.5 µL reverse primer. Vortex then spin mixture in mini-microcentrifuge, then keep on ice.
3. Add 24 µL of master-mix to 1 0.2 mL PCR tube per reaction.
4. Add 1 µL of DNA to each tube. Use 1 µL nuclease-free water for the negative control.
5. Spin tubes in the mini-microcentrifuge then place them in the thermal cycler. Run samples on an amplification program which should have the following conditions:
    a. 16 cycles of the following:
        i. 95^{o}C for 10 seconds
        ii. 62^{o}C for 30 seconds, -1^{o} per cycle
        iii. 72^{o}C for 60 seconds
    b. 25 cycles of the following:
        i. 95^{o}C for 10 seconds
        ii. 46^{o}C for 30 seconds
        iii. 72^{o}C for 60 seconds
    c. Final hold at 4^{o}C
6. Samples can be frozen in a -20^{o}C freezer if not moving directly on to part E.

D. Gel Electrophoresis

Materials and Equipment:

- PCR products
- Invitrogen Low DNA Mass Ladder
- Pipettes and sterile, disposable pipette tips (ideally filter tips)

Large 1.5% Agarose Gel (Maximum 40 wells):

- 150 mL TBE Buffer
- 2.25 g Agarose
- 2.5 µL Ethidium Bromide

Small 1.5% Agarose Gel (Maximum 24 wells):

- 60 mL TBE Buffer
- 0.9 g Agarose
- 2.0 µL Ethidium Bromide

Procedure:

1. Combine TBE buffer and agarose in a flask.
2. Heat in a microwave in 30 or 60 second intervals depending on if making a small or large gel. Heat until mixture boils and appears clear.
3. Cool flask until it can be touched for three seconds on any side.
4. Add ethidium bromide and gently swirl mixture. Pour into gel rig. Let sit at least half an hour.
5. Pipette 5 µL of Invitrogen Low DNA Mass Ladder into the first well. Pipette 15 µL of each PCR product into its own well, making sure to include the negative control.
6. Run the gel for 30 minutes at voltage specified above then view using a UV transilluminator with a mounted camera. If you do not see bands for your PCR products, go back and troubleshoot your PCR. If your PCR products show bands, you can proceed.
7. Samples can be frozen in a -20^{o}C freezer if not moving directly on to part F.

E. Sample Clean-Up and Sequencing at the University of Maine's DNA Sequencing Facility

Materials and Equipment:

- Affymetrix ExoSAP-IT
- 0.2 mL microcentrifuge tubes
- 10 µM PCR primers
- Pipettes and sterile, disposable pipette tips (ideally filter tips)
- UV Transilluminator

Procedure:

1. Add 2 µL of Affymetrix ExoSAP-IT to one 0.2 mL microcentrifuge tube for each PCR product. Keep tubes on ice.
2. Add 5 µL of a PCR product to each tube and spin briefly in a mini-microcentrifuge.

3. Place tubes in a thermal cycler and run on the ExoSAP program. The product can either be frozen in a -20^{o}C freezer or you can proceed.
4. In a new 0.2 mL microcentrifuge tube, dilute 1 µL of each PCR product with enough nuclease-free water to yield a final DNA concentration of 10 ng/µL.
5. Briefly spin tubes in the mini-microcentrifuge and deliver to the sequencing lab.

F. Sample Identification

Procedure:

1. Once sequence files returned, typically in ".abi" format, open them in a DNA editing software (e.g. 4Peaks for Mac OSX) then:
    a. Visually scan sequences for sequence quality.
    b. Trim the ends to remove poor sequence quality.
    c. For sequences from the reverse primer, "Flip Sequence" (reverse complement).
    d. Highlight and copy (ctrl-c) sequence.
2. Go to the Barcode of Life Database (http://boldsystems.org) and click on the "Identification" tab.
3. While under the "Animal Identification [COI]" tab, paste (crtl-v) your sequence into the text box.
4. If no match is found, repeat selecting less stringent search options.
5. Check that the search results for the forward and reverse sequences from each specimen match.

Note: All standard lab safety procedures should be followed throughout, including, but not limited to, use of safety glasses or goggles, gloves, and other personal protective equipment; the separation of the sterilizing flame from areas in which ethanol is being used; and storage of reagents and chemicals in an appropriate manner.

AUTHOR'S BIOGRAPHY

Megan Little was born in Bangor, Maine on May 31, 1995. She was raised in Calais, Maine and graduated valedictorian of her class from Calais High School in 2013. Megan is a double major in molecular and cellular biology and biochemistry. In the fall she will continue her education at Marshall University working towards obtaining a Master's degree in forensic science with a concentration in DNA analysis.