Analysis of mtDNA of Teneral Odonate Species Between Two Ponds Brookhaven, New York: A Model for High School Research

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Abstract

Understanding how individuals in a population are related to each other on an evolutionary scale can be achieved by analysis of mitochondrial DNA (mtDNA). Amplification primers for this project were identified by Shaw (1996) from *Drosophila yakuba.* All Odonate samples were captured in July 2009 at two ponds located at Brookhaven National Lab (BNL). All laboratory techniques and sequencing were conducted at BNL. Teneral species showed three distinct polymorphisms. This species has not been entered into GenBank and could not be positively identified through a BLAST search. This project promotes questions as the foundation for additional research for high school students.

Introduction

Understanding how individuals in a population are related to each other on an evolutionary scale can be achieved by analysis of mitochondrial DNA (mtDNA). Results may allow for inferences to be made regarding habitual behavior and migratory pathways as well as to determine relatedness to each other and other species within the Odonates.

Previous studies have been conducted on insects to identify how closely related individuals within a population are. Saux, Simon, and Spicer (2003), studied mtDNA sequence data to investigate the relationships of the Odonata across both the Anisoptera (dragonflies) and the Zygoptera (damselflies). Amplification primers were identified by Shaw (1996) from *Drosophila yakuba* and correspond to mtDNA sites 14588-14612 (12Sai, 5' –AAA CTA GGA TTA GAT ACC CTA TTAT) and site 14214-14233 (12Sbi, 5' –AAG AGC GAC GGG CGA TCT GT) (Saux, Simon, &Spicer, 2003). This investigation aims to confirm the classification of teneral Odonates found at two ponds located at BNL, to determine whether rare haplotypes can be identified, and to generate a phylogenetic tree of closely related species.

Weaver Pond
Photo taken by M. Brown, 2009

Half Moon Pond
Photo taken by M. Brown, 2009

Methods and Materials

Field Collections:
Telescoping soft mesh insect nets were used to capture teneral Odonates as they were emerging from Half Moon and Weaver Ponds between 9 – 11 am. They were placed temporarily into a butterfly pavilion until catalogued and then frozen.

Emerging teneral Odonate
Photo taken by M. Brown, 2009

DNA Extraction:
Odonate DNA was extracted using a Qiagen Dneasy ® Blood and Tissue Kit for a total of 60 dragonfly of which 54 were tenerals.

Photo taken by M. Brown, 2009

Polymerase Chain Reaction (PCR):
Taq Polymerase was added to DNA samples and a master mix of 22.5 µl primer mix, dNTPs, Mg++, and buffer into a 96 sample well plate. The PCR machine ran for 34 cycles at 94 °C denaturing for 20s, 52 °C primer annealing for 30s, and 68 °C primer extension for 1-minute.

Analysis of PCR:
15 µl of PCR-ready sample was transferred into a 2% agarose gel (E-gel ®) for each test specimen. The remaining volume of sample was saved for sequencing.

DNA Purification/Sequencing:
DNA was purified for sequencing using 150 µl of 50% guanadine to denature the proteins and 200 µl of 80% ETOH. A NanoDrop spectrophotometer was used to identify the density of DNA (ng/µl) in samples that showed strong, light, and no electrophoresis gel bands. Prior to sequencing, DNA were prepared using an ethanol precipitate reaction, ice, and centrifuge to rid the DNA of salts and dye terminators from the sample. Samples were sequenced for 16-hours.

PCR
Photos taken by D. Williams, 2009

NanoDrop Spectrophotometer
Photo taken by M. Brown, 2009

Results and Discussion

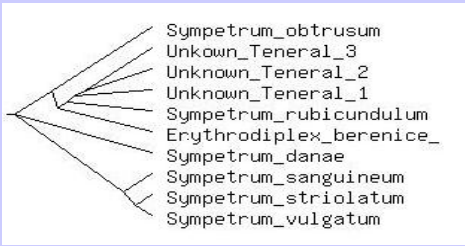

Figure 1: Bioservers Phylogenetic Tree (D. Williams, 2009)

Figure 2: Clustal W Sequence Alignment (D. Williams, 2009)

Sixty Odonate samples prepared for PCR and gel electrophoresis were analyzed. Eighteen samples (30%) were removed prior to sequencing as a result of PCR (no bands observed) and NanoDrop spectrophotometer analysis (unsuitable DNA density). A total of 43 samples (13.95%) were sequenced included a positive control (*Erythrodiplax berenice*) using a Hitachi ABI Prism 3130xl Genetic Analyzer.
Sequencher was used to trim, analyze, and produce forward and reverse chromatograms. Five distinctly different groups were identified :

1. "Teneral 1" 17 individuals – 41.18% Weaver Pond / 58.82% Half Moon Pond
2. "Teneral 2" 7 individuals – 42.86% Weaver Pond / 57.14% Half Moon
3. "Teneral 3" 2 individuals – 50% Weaver Pond / 50% Half Moon Pond
4. The positive control; seaside dragonlet *(Erythrodiplax berenice)*
5. Sample 60, ruby meadowhawk *(Sympetrum rubicundulum)*

A basic local alignment search tool (BLAST N) was conducted through the National Center for Biotechnology Information (NCBI) in an effort to identify the Odonate teneral species. There were no matches at 100%. Phylogenetic trees were configured with the closest matches found in GenBank. Further analysis and phylogenetic tree configuration (Figure 1) was accomplished using Bioservers Clustal W Alignment from the DNA Learning Center hosted by Cold Spring Harbor Labs. The results of the analysis indicate that Tenerals 1, 2, and 3 are closely related and are likely to exhibit three distinct polymorphisms within the same species. The unknown teneral Odonate is closely related to *Sympetrum rubicundulum* and *Erythrodiplax berenice.*

Conclusion:

Limited Odonate sequencing data has been added to GenBank for species in the northeastern United States and limited the overall analysis of this study. Further studies should include additional analysis of teneral species found at both Weaver and Half Moon Ponds since all three polymorphisms exist in individuals from both sites. Additional sites in Suffolk County should be investigated as well to determine if additional polymorphisms exist within the species. Capture of the teneral species should be conducted to allow individuals to develop adult coloration for positive identification. Thereafter, sequencing data for this species should be added to GenBank. Additional studies using 28S and 16S rDNA should be considered as outlined by Hasegawa & Kasuya (2006). The opportunity for high school students to conduct this type of research is promising. It provides a mechanism to conduct authentic research while adding important information to the GenBank database. It also provides a means of learning molecular biology techniques as applied to molecular ecology, while promoting scientific inquiry and a foundation for formulating new questions for further research.

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