Using Microsatellite DNA to Analyze Population Structure in Spotted Turtles

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Abstract
Blood samples were collected from spotted turtle populations on Nantucket and in Halifax, MA. DNA was extracted using QIAamp DNA extraction kits and was amplified by polymerase chain reaction using 7 microsatellite loci primers developed for bog turtle (Glyptemys muhlenbergii). The lengths of amplified microsatellite fragments were determined for each turtle using Beckman CEQ8000 sequencer to determine allele frequency. Allele length of the individual samples was used to determine heterozygosity, effective population size, rates of inbreeding, and migration within and between the mainland and island populations. While some loss of genetic diversity in the Nantucket population was indicated, highly significant variation was still found within and between the Nantucket and Halifax populations.

Methods

**Blood Spots collected**

**DNA Isolation**

QIAamp DNA extraction mini kit

**PCR with non-fluorescent primer pairs**

1.5% agarose gel electrophoresis to check for amplification in sample

PCR with fluorescent primer pairs

1.5% agarose gel electrophoresis to check for amplification

CEQ8000 sequence to determine allele size

Genepop

**Gene pop - Genetic population analysis**

**Primer sequences**

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<tr>
<th>Primer</th>
<th>Length (bp)</th>
<th>Note</th>
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<tr>
<td>D16</td>
<td>132</td>
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<td>D10</td>
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<td>D12</td>
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Observed and expected heterozygosity were calculated by Genepop using the Hardy-Weinberg model. Microsatellites with discrepancies between the values of H₀ and Hₑ were found to have high null allele frequencies.
Background

The spotted turtle (Clemmys guttata) is a small, semiaquatic species that is endemic to the eastern seaboard and the Great Lakes region of the United States. Due to habitat alteration and fragmentation, population declines and isolation are becoming prevalent issues throughout the species’ range. Spotted turtles were previously listed as a species of special concern in Massachusetts and a comprehensive genetic study of the spotted turtles on Nantucket Island, especially compared to mainland populations, might provide critical information about the genetic diversity of the species because it has been isolated from mainland populations for many generations. For the purposes of this initial study, 40 turtles were sampled from the Medouie Creek Wetland Complex on Nantucket and 10 turtles were sampled from Halifax, MA.

Microsatellites are tandem repeats of less than six base pairs that exhibit high rates of polymorphism due to single strand slippage during replication. Due to this polymorphism, analysis of microsatellite loci length in individuals within a population can be used to estimate effective population size, determine heterozygosity in a population, and understand genetic relationships among populations.

Objectives

- Use polymerase chain reaction to amplify microsatellite loci in Clemmys guttata
- Determine lengths of microsatellite loci alleles in individuals using Beckman CEQ8000 sequencer
- Determine the effective population size, heterozygosity, and inbreeding rates of Nantucket and Halifax populations.

Pairwise Fst

- Nantucket vs Halifax Populations = 0.068 Essentially, 6.8% of the observed genetic diversity is between the populations.
- Nantucket is 93.2% genetically distinct from the Halifax population.

Discussion

This study revealed that the Nantucket spotted turtle population is genetically distinct from Halifax populations and therefore should be treated as a separate management unit. Gene flow potentially occurred between these two populations but is likely no longer occurring. Despite lower genetic diversity and a small effective population size, sufficient genetic variation still exists among individuals in Nantucket’s Medouie Creek spotted turtle population.

Future directions for this project include increasing sample size from the Halifax population, collecting additional samples from other mainland populations and comparing the Medouie Creek spotted turtles to a population on the west side of Nantucket with the goal of determining gene flow across the island.

References


Acknowledgments

I would like to thank Dr. Barbara Brennessel and the Wheaton College Department of Biology for their support on this project and Danielle O’Dell and Dr. Charles Innis for collecting blood samples. Funding for this project was provided by the Nantucket Conservation Foundation and the Nantucket Conservation Trust.

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