Examining Mitochondrial Genetic Diversity in a Population of Eastern Hognose Snakes in Cape Cod, MA

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Abstract

The Eastern Hognose snake (*Heterodon platirhinos*) is a relatively poorly studied species found in the eastern half of the United States from southern New England and Ontario south along the Atlantic coast to Florida and west to Texas, Kansas, Nebraska, and South Dakota. In the Northeastern part of their range they are considered to be a species of regional conservation concern by the Northeast Endangered Species and Wildlife Diversity Technical Committee, and are protected by conservation measures in the states of Rhode Island, Connecticut, and Massachusetts, and are listed as endangered by the state of New Hampshire. The purpose of this study was to examine the genetic diversity of a population of *H. platirhinos* in Cape Cod National Seashore, Barnstable, MA in order to determine whether this population was being impacted by habitat fragmentation. Tissue samples were collected from snakes in conjunction with a radio telemetry study which covered three major towns in the northernmost part of the peninsula of Cape Cod. DNA was obtained from a total of twenty-three snakes and compared partial sequences of the mitochondrial control region.

Introduction

The Eastern Hognose snake (*Heterodon platirhinos*) is considered to be a species of regional conservation concern by the Northeast Endangered Species and Wildlife Diversity Technical Committee in the Northeastern part of their range (Therres 1999). Populations of many North American snake species have declined due to factors such as habitat loss or modification (Greene 1997, Gibbons et al. 2000, Lagory 2009). Our understanding of snake spatial ecology and habitat use has improved over the past several decades with the utilization of radiotelemetry studies (Fitch and Shirer 1971, Prior and Weatherhead 1996, Reinert 1984). For *Heterodon platirhinos,* however, relatively few quantitative ecology studies have been compiled (Plummer and Mills 2000, Lagory 2009), and none have explored any aspect of the species' genetic history. Molecular techniques for snake conservation planning and population ecology have also grown in recent years. Mitochondrial DNA analysis can assist with long term planning and with implementing short-term goals of species recovery plans (Moritz, 1994).

Methods

- DNA was extracted from samples using QIAmp Mini Kit (QIAGEN)
- PCR reactions were performed in 25 µl reactions using 12.5 µl DNA, 1X PCR Buffer, 2.0 mM $MgCl_2$, 0.2 mM DNTP, 0.4 µM of each primer, 6.5 µl H_2O, and 0.1 U/ µl Taq.
- The primers H690 (GTT GAG CCT TGC ATG TAT A) and L16090 (TAA AGC ATT GTT CTT GTA AAC CAA AG) were used (Burbrink et. al 2000, Kumazawa et al 1996).
- PCR reactions were performed using a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA).
- PCR products were verified using gel electrophoresis to confirm amplification and to indicate that the reactions were not contaminated.
- PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) and sequenced using an ABI3100x automated sequencer.
- All sequences obtained were aligned using the program MUSCLE (MUltiple Sequence Comparison by Log- Expectation) (EMBL-EBI).
- Variable base pairs were confirmed by visually analyzing chromatogram files for signal strength and quality of base pair calls.
- Haplotypes were identified and statistics were generated using DnaSP
- Statistical parsimony analyses were performed using the program TCS 1.21

Study Site

Cape Cod incorporates all of Barnstable County, which comprises fifteen towns.. Three of these towns are represented in this study: Wellfleet, Truro, and Provincetown. These three major towns in Cape Cod cover approximately 120 sq km of the northernmost part of the peninsula.

Results

DNA was obtained from a total of twenty-three snakes and partial sequences (151 bp) of the mitochondrial control region were compared. Of the twenty-three snakes, four were collected in Truro, six in Wellfleet, and thirteen in Provincetown. All sequences were joined in a statistical parsimony network with a 95% confidence connection limit (Fig. 1). Five unique haplotypes were distinguished which differed by no more than three base pairs. A total of seven mutations exists among all individuals, all with only two variants, at sites 22; 54; 66; 72; 80; 95; 119 (Fig. 3). Nineteen individuals expressed Haplotype 1, while Haplotypes 2, 3, 4, and 5 were only expressed by one individual each (Fig. 1). All three locations (Truro, Provincetown, Wellfleet) held individuals with Haplotype 1. Individuals from Truro were found to have Haplotypes 1, 2, 3, and 4. The individual with Haplotype 5 was from Provincetown, however individuals from Provincetown were found to also have Haplotype 1. All Wellfleet individuals fell into Haplotype 1 (Fig. 2). Haplotype diversity (Hd) was found to be 0.324 and nucleotide diversity (Pi) was found to be 0.00403.

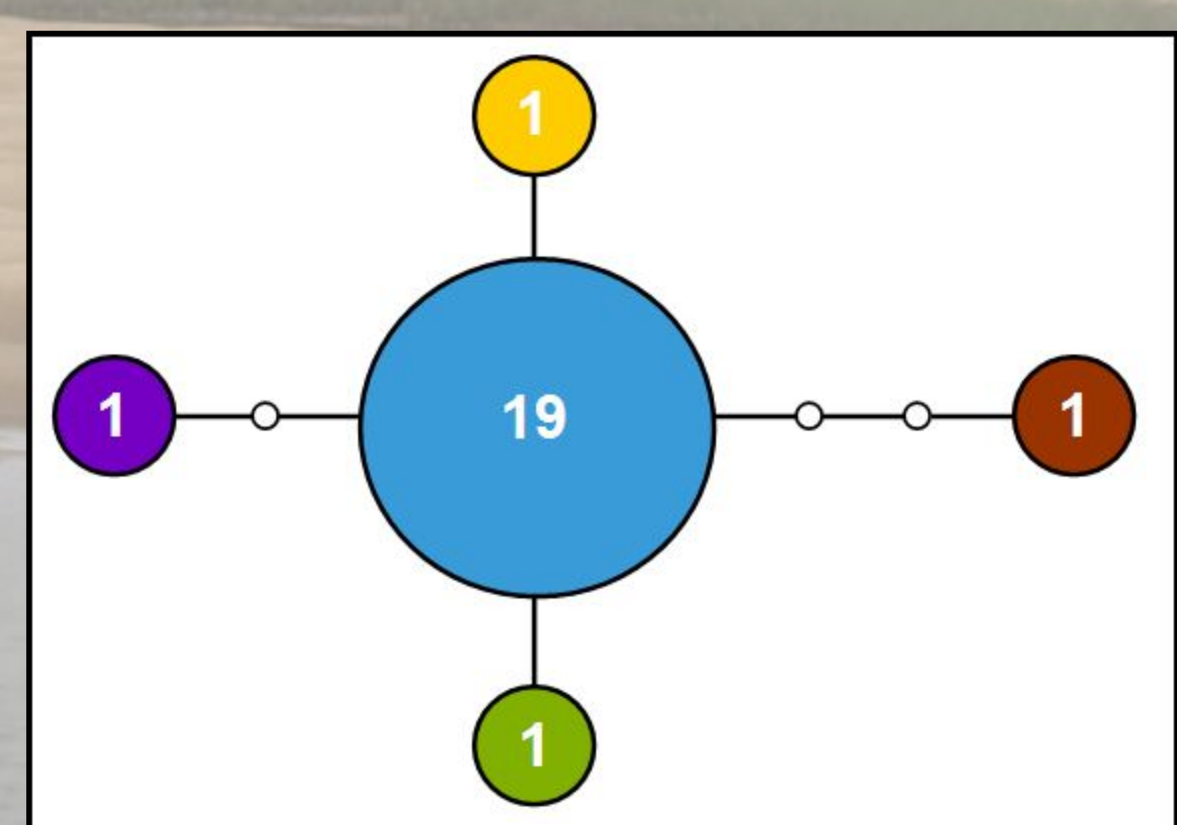

Fig. 1. Statistical parsimony network of twenty-three *Heterodon platirhinos* connected at a 95% significance level. White dots represent mutational steps and the number represents the number of individuals expressing each haplotype.

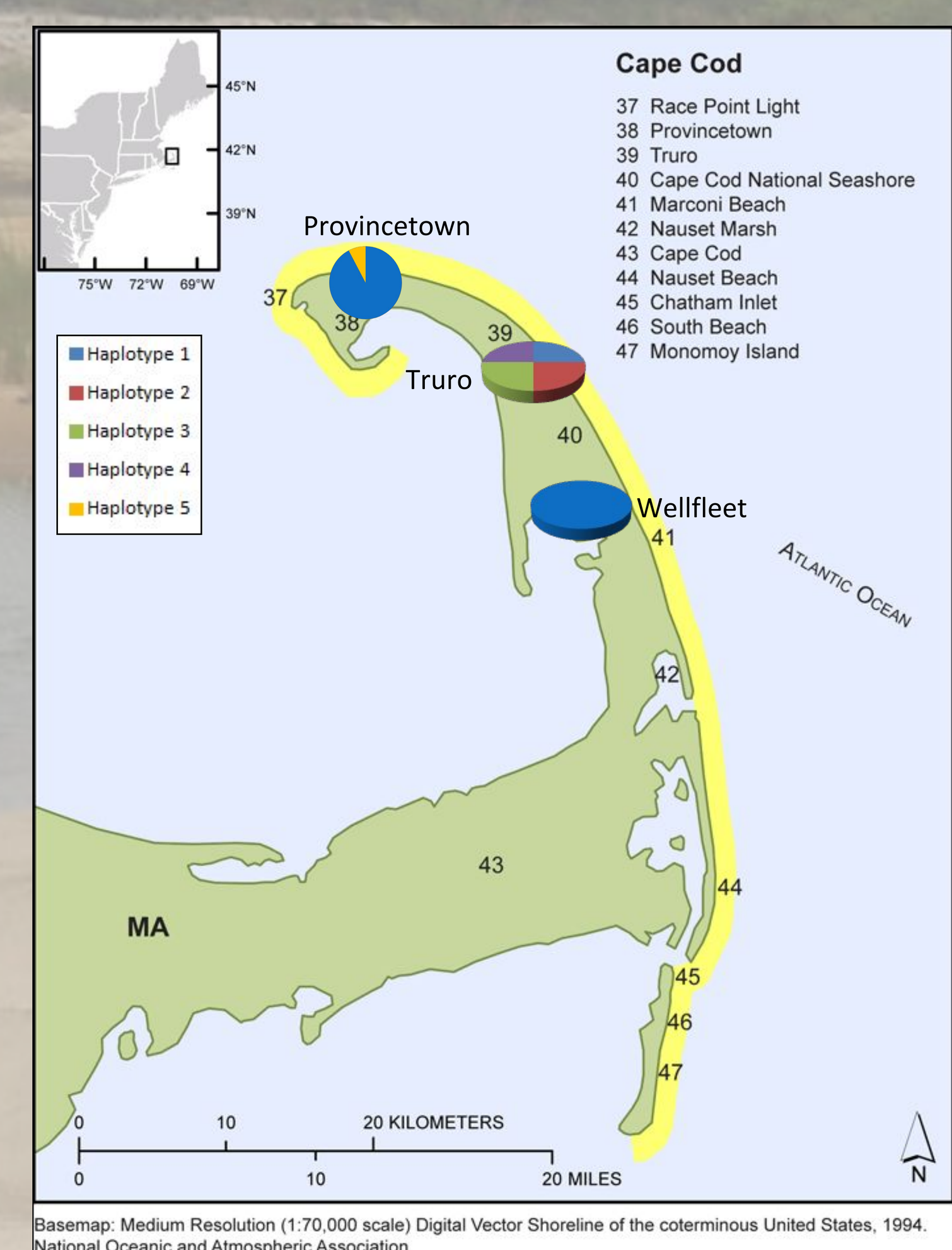

Fig. 2. Distribution of haplotypes across each of the study site locations.

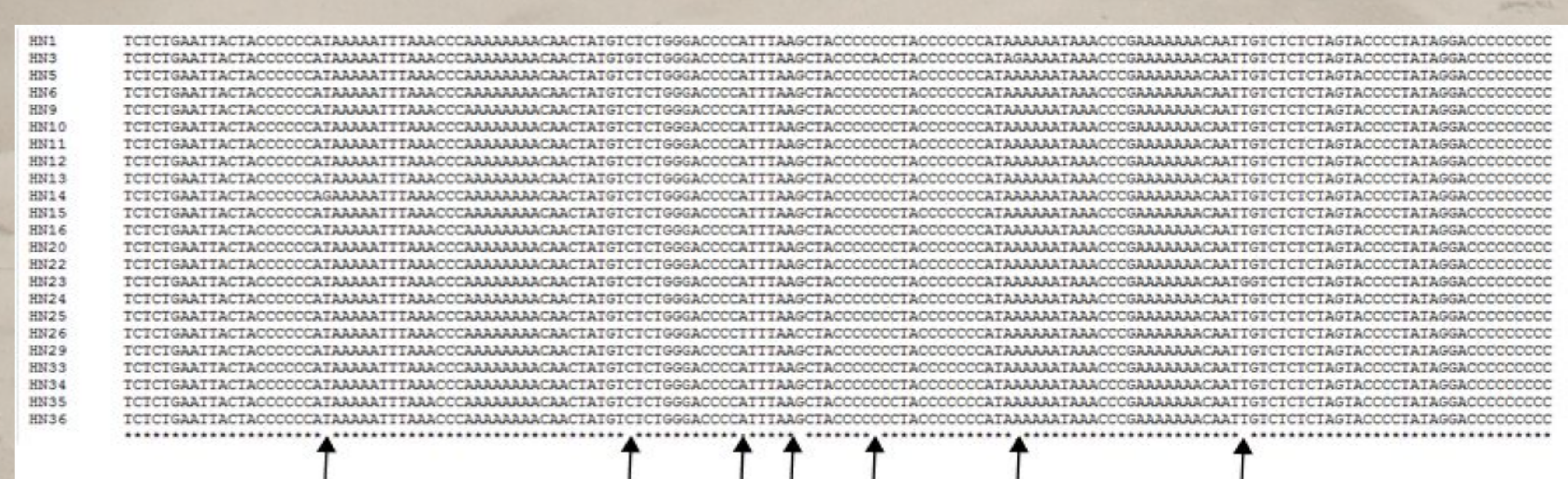

Fig. 3. Five unique haplotypes out of the twenty-three individuals, with seven mutations indicated above by an arrow.

Discussion

Across all individuals, haplotype diversity was moderate, and nucleotide diversity was quite low (Hd = 0.324, Pi = 0.00403). The hypothesis that the farthest sites would have less similar populations was not supported. Interestingly, the central population, Truro, was found to have three individuals with private alleles. This suggests that this population may be isolated from those North and South of it. This isolation is most probably recent because the haplotypes only differ by one and two substitutions.

Telemetry data that were collected in conjunction with this suggest that these three towns may contain three separate populations. Estimates of daily movements averaged 25.9 m/d and were less than estimates derived from other studies of *H. platirhinos*. Smaller home ranges may be attributed to a barrier to movement.

Future Research

Future research will include more individuals from all of the locations. Furthermore, microsatellites should be developed in order to distinguish finer resolution of population genetic parameters and patterns not shown by mitochondrial DNA.

Acknowledgments

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http://flickrhivemind.net/Tags/platirhinos

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