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Examining Mitochondrial Genetic Diversity in a Population of Eastern Hognose Snakes (Heterodon platirhinos) in Cape Cod, MA

Stephanie R. Parelli

ABSTRACT

Examining Mitochondrial Genetic Diversity in a Population

of Eastern Hognose Snakes (*Heterodon platirhinos)* in Cape Cod, MA

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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. They 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, MA, in order to determine whether this population was being impacted genetically 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 partial sequences of the mitochondrial control region were compared. All sequences were joined in a statistical parsimony network with a 95% confidence connection limit. Five unique haplotypes were distinguished which differed by no more than three base pairs, and there was no correlation between geographic location and haplotype occurrence. These results suggest

that this population in Cape Cod may not experience any barriers to gene flow or that barriers may have been recently established. To my knowledge, this is the first genetic analysis of the Eastern Hognose snake.

MONTCLAIR STATE UNIVERSITY

Examining Mitochondrial Genetic Diversity in a Population

of Eastern Hognose Snakes (*Heterodon platirhinos)* in Cape Cod, MA

by

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EXAMINING MITOCHONDRIAL GENETIC DIVERSITY IN A POPULATION OF EASTERN HOGNOSE SNAKES (*HETERODON PLATIRHINOS*) IN CAPE COD, MASSACHUSSETTS

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CHAPTER I

INTRODUCTION TO THE GENETICS AND CONSERVATION OF SNAKES

Genetics and Conservation

The Eastern Hognose snake (*Heterodon platirhinos*) is listed as a species of regional conservation concern by the Northeast Endangered Species and Wildlife Diversity Technical Committee (Therres, 1999) in the Northeastern part of their range. Populations of many North American snake species have declined due to factors such as habitat loss or modification (Dodd, 1987; Greene, 1997; Gibbons *et al.*, 2000; Lagory, 2009). The effects of habitat fragmentation on a population can be better understood using field-based techniques geared towards spatial ecology such as radiotelemetry, as well as with molecular techniques such as DNA analysis. Increasing numbers of studies now use variation in mitochondrial DNA to assist with demographic studies of populations, especially those which are threatened or managed.

Mitochondrial DNA analysis can provide signals of population changes that otherwise would be time-consuming, expensive, and difficult to perform via direct studies. Mitochondrial DNA analysis can assist with long-term planning and with implementing short-term goals of species recovery plans (Moritz, 1994). Mitochondrial DNA has been successfully used to illustrate genetic and geographic patterns in numerous taxa including turtles (Starkey *et al.*, 2003; Amato *et al.*, 2007), salamanders (Zamudio and Savage, 2003), and snakes (Fontanella *et al.,* 2008, Guiher and Burbrink, 2008). These studies have all utilized mitochondrial DNA as mitochondria are haploid

and uniparentally inherited, which gives an effective population size one-fourth the size of the effective population size of nuclear genes (Moore, 1995). In addition, because of fewer DNA repair mechanisms, rates of substitution are higher in mtDNA than in coding regions of nuclear DNA so patterns of genetic divergence are evident in mtDNA before they may be seen in nuclear sequences (LeDoux *et al.,* 1992).

Genetic studies can provide a wide range of information such as population genetic structure, evolutionary relatedness among populations, direction and magnitude of gene flow, and levels of genetic diversity and variability within populations. These types of data are essential for developing effective management plans for animals likely to be affected by habitat fragmentation such as northeastern populations of *H. platirhinos.*

Rationale and Project Overview

Noted declines of reptiles are believed to be the result of direct anthropogenic pressures including habitat loss and fragmentation, road mortality, environmental degradation, and intentional killing (Ernst and Ernst, 2003; Gibbons *et al.*, 2000). Populations of many North American snake species in particular have declined due to factors such as habitat loss or modification (Dodd, 1987; Greene, 1997; Gibbons *et al.*, 2000; Lagory, 2009). Knowledge of habitat use is critical to snake conservation (Lagory, 2009) however, the greatest constraint in conservation planning for snakes is the lack of basic biological information for most species (Dodd, 1987; Dodd, 1993; Reinert, 1993; Lagory, 2009; Buchanan, 2012). Successful conservation planning is only achievable

through understanding specific biological constraints on a given species (Dodd and Seigel, 1991; Scott and Seigel, 1992).

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 (Therres, 1999) in the Northeastern part of their range, 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 (New Hampshire Natural Heritage Bureau, 2009). Massachusetts Division of Fisheries and Wildlife are currently trying to obtain further information about the species to better assess its status in Massachusetts (Buchanan, 2012).

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; Sperry and Weatherhead, 2009; Breininger *et al.*, 2012; Row, 2012). Molecular techniques for snake conservation planning and population ecology have also grown in recent years. 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.

Genetic Consequences of Habitat Fragmentation in Snakes

The genetic consequences of habitat fragmentation and loss can affect species with different life histories in unique ways, as demonstrated by several recent studies.

Some species may modify habitat-use patterns (Githiru *et al.,* 2007) or be better adapted to moving through a fragmented landscape (Marchesan and Carthew, 2008). Other species may be strictly limited to certain habitat types resulting in isolated populations in fragmented landscapes with little to no gene flow between them (Greenwald *et al.,* 2009). For many species we have little information regarding specific habitats that influence occupancy. Effective management and conservation are hindered by a lack of basic natural history information and the small number of large-scale studies designed to assess general population trends.

An increasing number of studies have attempted to assess snake population subdivision based on genetic data in areas affected by habitat loss and degradation. Jansen *et al.* (2008) genotyped 125 individuals from seven locations along the Florida coast at four microsatellite loci of the mangrove salt marsh snake (*Nerodia clarkii compressicauda*) which occupies a unique and disappearing habitat in much of coastal southern Florida due to coastal real estate development. Populations of *N. c. compressicauda* appear to be fractured into isolated neighborhoods due to extensive habitat fragmentation and high predation pressure in open spaces (Jansen *et al.,* 2008).

Meister *et al.* (2012) used seven microsatellite markers to examine the genetic population structure of the Grass Snake, *Natrix natrix*, from three different areas which were 30-100 kilometers apart. The three study sites were varied in habitat type (remnants of a pristine habitat in a former wetland in the Swiss lowlands, a rural valley in the Alps, and an intensively used agricultural area) but were interconnected by the river Aare. At the local scale, no genetic differentiation was found in either of the *N. natrix* populations

inhabiting the rural alpine valley or the agricultural area, however, two subpopulations in the former wetland area were genetically differentiated with a low but significant measure of genetic differentiation between subpopulations. This slight genetic differentiation can be explained by isolation by distance. On a regional scale they found significant genetic differentiation between *N. natrix* populations inhabiting areas separated by 30–100 kilometers. The genetic structure was also highly related to isolation by distance. According to this study, the genetic structure of Grass Snakes is mainly affected by geographic distance, while human activity and habitat alteration do not seem to reduce the snakes' movements (Meister *et al.,* 2012).

Prior *et al.* (2003) found evidence of significant genetic structure among black rat snakes (*Elaphe obsoleta)* sampled at three spatial scales using randomly amplified polymorphic DNA markers. Highly isolated (1500–1900 km apart) populations were strongly divergent, whereas populations more proximal, although currently isolated, exhibited far less divergence. At the scale of sub-populations (local populations 15–50 km apart), differentiation was generally moderate. Prior *et al.* (2003) also found that habitat type affected genetic structure. A pair of hibernacula sampled in an urban area exhibited genetic structure equivalent to some sub-population differences which suggests interrupted gene flow related to urban development (Prior *et al.,* 2003).

The loss and fragmentation of pristine habitat restrict specialized species to remnants of original habitat patches in a less suitable landscape. This may lead to a genetic differentiation of the subpopulations and to a decline in biodiversity. Distance also seems to be a factor in isolation and may be a larger component of genetic

differences based on geographic characteristics. Understanding how individuals and species move through landscapes is also essential for predicting impacts of landscape alterations (Row *et al.*, 2010; Gibbs *et al.,* 1997). Information on dispersal patterns, however, is lacking for many taxa, particularly snakes. A study by Row *et al.* (2010) combined habitat suitability modeling with population genetic analyses to infer how Eastern foxsnakes (*Mintoinus gloydi*) disperse through a habitat mosaic of natural and altered landscape features. Their results suggest that habitat degradation limits dispersal for foxsnakes, which has had a strong effect on the genetic population structure throughout the fragmented region. DiLeo *et al.* (2010) investigated the effects of a fragmented landscape on the genetic population structure of two sympatric snake species that differ in habitat preference, the eastern garter snake (*Thamnophis sirtalis sirtalis*) and the endangered eastern foxsnake (*Mintonius gloydi*). These species differ in habitat preference, and the eastern garter snake is a common snake and a habitat generalist whereas the eastern foxsnake is rare, and a marsh-specialist. Results of this study show that unsuitable intervening habitat such as agricultural tracts and roads between existing populations of foxsnakes appears to act as barriers to gene flow, while garter snake movement appears unrestricted by these features (DiLeo *et al.,* 2010).

Gibbs *et al.* (1997) looked at six microsatellite loci in 199 individuals from five populations in Ontario, New York and Ohio to better understand the degree of genetic differentiation between, and the levels of inbreeding within populations of the eastern massasauga rattlesnake (*Sistrurus c. catenatus*). The results of this study suggest that due to limited dispersal, populations may be genetically differentiated even within continuous populations (Gibbs *et al.,* 1997). Inbreeding due to isolation can cause a lower degree of

genetic heterozygosity due to fixation or near-fixation of alleles, and a higher genetic similarity among individuals (Madsen *et al.,* 1996). Madsen *et al.* (1996) looked at an isolated population of snakes (*Vipera berus*) that has been separated from neighboring populations by the expansion of agricultural activities in southern Sweden. Total adult population size is < 40 adult individuals, and the mating system is such that a few males have disproportionate reproductive success and therefore father most of the offspring each year. Inbreeding is promoted by the isolation and small effective population size (< 15 adults). In addition to a lower degree of genetic heterozygosity among individuals, compared to other non-isolated Swedish populations of adders, the isolated population also showed a smaller litter size relative to maternal body size and a higher proportion of deformed and stillborn offspring, traits often related to inbreeding depression (Madsen *et al.,* 1996).

Current studies suggest there are many possible genetic consequences of the effects of habitat fragmentation and anthropogenic land use on snakes. Despite the increase in these types of studies, there are currently very few ecological studies of the Eastern Hognose snake and no genetic data available for this species. Here, I describe the first genetic study of *H. platirhinos,* a phylogeographic study using mitochondrial DNA.

CHAPTER II

MITOCHONDRIAL GENETIC DIVERSITY OF THE EASTERN HOGNOSE SNAKE (HETERODON PLATIRHINOS) IN CAPE CODE, MASSACHUSETTS

Introduction to the Eastern Hognose Snake (*Heterodon platirhinos)*

Natural History, Distribution, and Status

The eastern hognose snake can be locally common in a variety of dry open habitats (woodland, forest edge, and sand barrens) with sandy well-drained surface soils (Platt, 1969; Ernst and Ernst, 2003). In New Hampshire, the main factor leading to the loss of Eastern Hognose Snake populations is the destruction or modification of suitable dry sandy habitats (New Hampshire Fish and Game Department, 2005). The Eastern Hognose snake also requires nearby wetlands habitat as their diet consists mostly of American toads (*Bufo americanus*). Additionally, other wetland-breeding amphibians contribute to 20-30% of their food source. They also require a supply of small amphibians, either salamanders, *Plethodon cinereus*, or peepers, *Hyla crucifer*, to sustain hatchlings and young until they are large enough to eat toads, as *B. americanus* do not metamorphose into the terrestrial form until late summer (Michener and Lazell, 1989). Multiple studies have indicated that *H. platirhinos* are predominantly diurnal (Platt, 1969; Plummer and Mills, 2000). *Heterodon platirhinos* are found among a variety of habitats; however they occur in greatest densities in well drained, sandy soils and barrier beach and dune ecosystems (Brady, 1925; Conant, 1938; Scott, 1985; Stewart and Rossi, 1981; Fitch, 1993). Throughout most of its range, *H. platirhinos* appear to occur in low population densities relative to sympatric snake species (Fitch, 1993; Ford, 1991).

Studies have measured body temperatures and mean nest temperatures in the natural environment, and basking appears to be important in the thermal ecology of *H. platirhinos* (Cunnington and Cebek, 2005; Plummer, 2010). Body temperature ranged from 5° to 37°C and varied with air temperature and activity (Plummer, 2010). Mean nest temperatures in the field ranged from 23.4° to 26.1°C, while incubation periods ranged from 49 to 63 days (Cunnington and Cebek, 2005). Trailing has been found to be used by male *H. platirhinos* to locate females and that the increased frequency and distance of movement by males increases the chance of locating the trail of a female (Plummer, 1996). Generation time for *H. platirhinos* is not clearly defined; however they are estimated to have a generation time between four and eight years (Buchanan pers. comm.). *Heterodon platirhinos,* as well as other species within the *Heterodon* genera, have been the focus of relatively few studies compared to other Colubrid snakes. The aforementioned studies have focused on physiology (Platt, 1969; Plummer, 2010), reproductive traits (Plummer, 1996; Cunnington and Cebek, 2005), spatial ecology (Plummer, 2000), or life history (Platt, 1969; Buchanan, 2012). To date, no analysis of genetic variation has been performed, leaving a large gap in knowledge for this species.

Materials and Methods

Study Site

Cape Cod, or Barnstable County, in southeastern Massachusetts is a long, narrow peninsula that extends out into the Atlantic Ocean and it includes many specialized habitats including Coastal dunes, maritime forests, Atlantic White cedar swamps, coastal

plain ponds, sandplain grasslands, estuarine intertidal marshes, coastal salt ponds, maritime oak/holly woodlands, and pitch pine/scrub oak woodlands. Due to the position of the peninsula jutting out into the Atlantic Ocean and the complete absence of surface bedrock, Cape Cod is naturally subjected to massive coastal erosion and deposition, however because of sea level rise it is predicted that the average natural erosion rate is approximately three feet a year (Giese and Giese, 1994).

Cape Cod incorporates all of Barnstable County, which comprises fifteen towns, three of which are represented in this study, Provincetown, Truro, and Wellfleet (Appendix A). The northernmost sites included in this study, Provincetown and Truro, are separated by approximately six kilometers of an unbroken patch of sand dunes, shrublands, and temporary wetlands which is an ideal habitat for hognose snakes and their prey (*Bufo americanus* and other wetland-breeding amphibians). Following World War II a large amount of housing development took place throughout Cape Cod, but was later limited within the borders of the Cape Cod National Seashore (CCNS). This created a small corridor on the east side of Truro as undeveloped land, but the western side of CCNS is heavily developed. The third study site, Wellfleet, is separated from Truro by approximately 16 kilometers, most of which is pine forest with very few wetlands capable of supporting hognose snake's food source (Appendix A). The population found in Wellfleet is primarily based around a number of kettle ponds, which are surrounded by oak and pine forests and coastal heathland, and development increases to the south of the kettle ponds.

Collection of Samples

Blood and tissue (scales and muscle) samples were provided by Scott Buchanan and other personnel from the Cape Cod National Seashore, and stored in Drierite desiccant prior to DNA extraction (Buchanan, 2012). All samples were identified by GPS coordinates of the location of collection, the date of the collection, and a code number representing the gender, length, and weight of the snake (Appendix B).

DNA Extraction

DNA was extracted from samples using QIAmp Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. DNA extracts were used as templates for the polymerase chain reaction (PCR), amplifying a portion of the mitochondrial control region. 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 to amplify this portion of the D loop and were originally designed for the North American rat snake (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). Negative controls were run using water in place of DNA. Amplification reactions began with an initial 7-minute denaturing step at 94° C. After the initial denaturation period, 40 cycles of amplification were performed using a 40 second denaturation step at 94° C, a 30 second primer annealing step at 45° C, and a 60 minute elongation step at 72° C. After the 40 cycles

were complete, the reactions underwent a final 7 minute elongation period at 72° C. PCR products were verified using gel electrophoresis to confirm amplification and to indicate that the reactions were not contaminated. Upon confirmation of amplification, PCR products were purified using QIAquick PCR Purification Kit (QIAGEN). Purified products were sequenced in both directions using the original PCR primers H690 and L16090 with an ABI3100x automated sequencer.

All sequences obtained were aligned using the program MUSCLE (MUltiple Sequence Comparison by Log- Expectation) (Edgar, 2004). Sequences were trimmed to remove peaks with weak signal and only higher quality peaks were used in the subsequent analyses. Variable base pairs were confirmed by visually analyzing chromatogram files for signal strength and quality of base pair calls. Haplotypes were identified using ClustalW2 (Larkin, 2007).

Sequence Analysis

Once variable haplotypes were identified, statistical parsimony analyses were performed using the program TCS 1.21, creating haplotype networks connected at a minimum 95% significance level (Clement *et al.*, 2000). DnaSP v5.10.1 was used to calculate haplotype diversity (Hd) and nucleotide diversity (Pi) (Librado and Rozas, 2009).

Results

Genetics of Heterodon platirhinos

DNA was obtained from a total of twenty-three snakes and partial sequences (151 bp) of the mitochondrial control region were compared. All sequences were joined in a statistical parsimony network with a 95% confidence connection limit (Fig. 1). Statistical parsimony confirms that there is a minimum 95% probability that any single mutational step between any two haplotypes only occurred once and therefore did not evolve multiple times independently. Five unique haplotypes were distinguished and no two adjacent haplotypes were separated by more than three base pairs (Fig. 2). Seven mutations exist among all individuals; mutations occurred at sites 22; 54; 66; 72; 80; 95; 119 (Fig. 2).

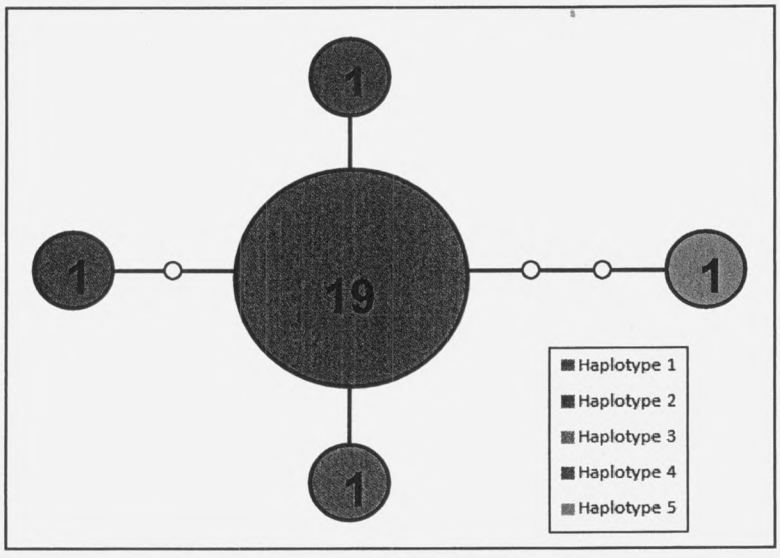

Figure 1. Statistical parsimony network of partial fragment of mtDNA DLoop from twenty-three *Heterodon platirhinos* connected at a 95% significance level. White dots and black vertical lines represent mutational steps and the number represents the number of individuals with each haplotype.

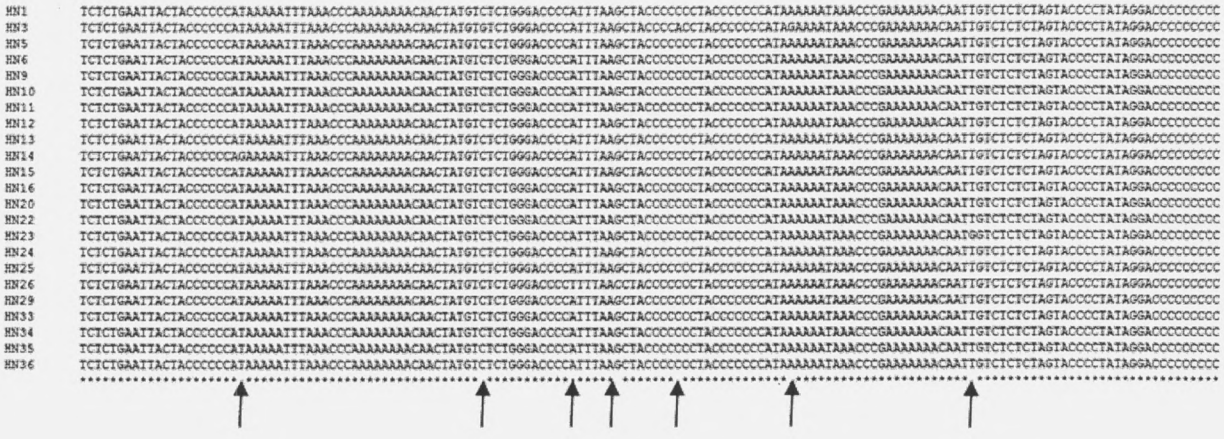

Figure 2. Five unique haplotypes of partial fragment of mtDNA DLoop from twenty-three *Heterodon platirhinos* , with seven mutations indicated above by an arrow.

Study sites and Genetics

Of the twenty-three snakes, four were collected in Truro, six in Wellfleet, and thirteen in Provincetown (Appendix A). The northernmost sites, Provincetown and Truro, are separated by approximately six kilometers of an unbroken patch of sand dunes, shrubland, and temporary wetlands which is an ideal habitat for hognose snakes and their prey. The third study site, Wellfleet, is separated from Truro by approximately sixteen kilometers, most of which is pine forest with very few wetlands capable of supporting hognose snake's food source (Appendix A). Haplotype 1 was found in 19 individuals, while Haplotypes 2, 3, 4, and 5 were only found in one individual each. Individuals from all three locations (Truro, Provincetown, and Wellfleet) had 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 had Haplotype 1 (Table 1). In

Wellfleet, 100% of individuals sampled were found to have Haplotype 1. In Truro, 25% of individuals sampled were found to have Haplotype 1, 25% with Haplotype 2, 25% with Haplotype 3, and 25% with Haplotype 4. In Provincetown, 92% of individuals were found to have Haplotype 1, and 8% were found to have Haplotype 5 (Fig. 3).

Location	# of individuals sampled Total	# of individuals with Haplotype 1	# of individuals with Haplotype 2	# of individuals with Haplotype 3	# of individuals with Haplotype 4	# of individuals with Haplotype 5
Provincetown	13	12	0	0	0	1
Truro	4	1	1	1	1	0
Wellfleet	6	6	0	0	0	0

Table 1. Number of *Heterodon platirhinos* individuals sampled at three locations on Cape Cod, MA and number of individuals with each haplotype for partial fragment of mtDNA DLoop.

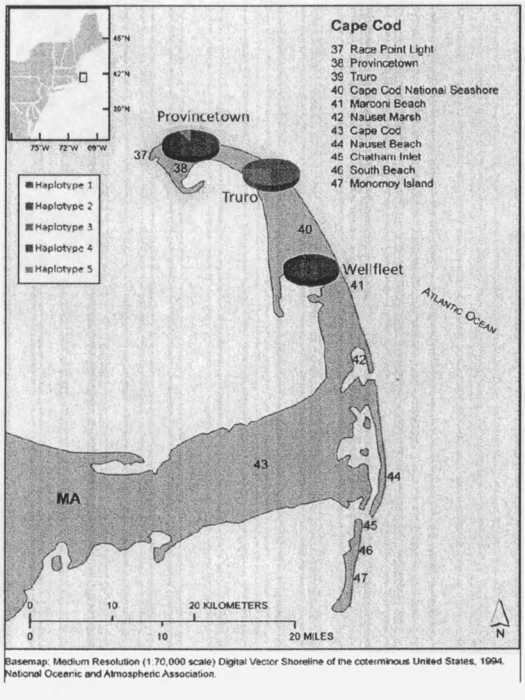

Figure 3. Distribution of *Heterodon platirhinos* haplotypes for partial fragment of mtDNA DLoop across each of the study site locations on Cape Cod, MA.

Discussion

Genetics of the Eastern Hognose Snake in Cape Cod, MA

Across all individuals, haplotype diversity was moderate, and nucleotide diversity was quite low (Hd = 0.324, Pi = 0.00403). The limited genetic variation within all three of these sites may be a sign that this population is isolated from other populations of *H. platirhinos*, the result of inbreeding, or a combination of both factors. Low genetic variation within populations could potentially have negative impacts, and limited genetic variation increases the probability of a population's extinction.

Populations within Cape Cod, MA

Telemetry data that were collected in conjunction with this genetic study 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*. The radiotelemetry study was limited to Provincetown, and found that although home range was large, and snake movements among snakes differed, the population stayed well within the borders of the Northern most point of the peninsula. Although the radiotelemetry study did not document individuals moving between Provincetown and Truro, it is likely that there are snakes throughout the large dune ecosystem which extends from Provincetown to the eastern side of Truro. The snakes which were tracked only represent a small sample of the population and most of the snakes went undetected. Additionally, sample collection for this study was not standardized in any way; therefore it cannot be assumed that the areas in which our

snakes were collected are the only areas between Truro and Provincetown capable of supporting *H. platirhinos*. Initially, this study distinguished three separate groups of snakes; a northern (Provincetown), middle (Truro), and southern (Wellfleet) population, in which the snakes were separated by the location of their capture for blood and tissue collection. However, there is a geologically distinct unbroken patch of sand dunes interspersed with temporary wetlands that stretch from Truro to Provincetown, so it may be more biologically meaningful to view these two populations as one connected location. There's no reason to think that there would be any significant isolation within this greater habitat. There is continuous ideal habitat for the snake as well as its prey between Provincetown and Truro with no obvious barriers to dispersal between these two sites. Consequently, snakes may be interbreeding between these two sites, an idea supported by the genetic data reported here, although not supported by the telemetry data. Given that Haplotype 1 was found in both areas, it is possible that snakes in both sites may be engaged in gene flow in a stepping-stone fashion. Stepping-stone gene flow could explain the discrepancy between the telemetry and genetic data. Although Haplotypes 2, 3, and 4 are private alleles restricted to Truro and Haplotype 5 is a private allele restricted to Provincetown, it is possible this is simply due to small sample size. If more snakes were sampled between these two points, these haplotypes may be discovered throughout the northern region. Due to the continuity of habitat and genetic data, I expect these two sites to be one intermingling population despite what the telemetry data suggest.

The southernmost study site, Wellfleet, is separated from Truro by approximately 16 kilometers, most of which is pine forest with very few wetlands capable of supporting

hognose snake's food source. If isolation exists between populations, I expect it would be between Truro/Provincetown (the northern population) and the population of snakes that are found in Wellfleet. Haplotype 1 is found in all three areas but is fixed in Wellfleet. This lack of genetic diversity in Wellfleet suggests this population may have recently become isolated from the northern part of this range and has not been separated long enough to accumulate new private alleles through mutation.

Future Considerations

A better understanding of whether fragmentation is having a long-term effect on gene flow can only be obtained through long-term telemetry and genetic monitoring. Future studies should aim to collect specimens for genetic testing from a wider geographic range. It is currently unknown whether hognose snakes live on the western side of Cape Cod, which is separated from this study's sites by a major road (Route 6). Museum samples collected from this area, as well as samples collected throughout their range should be included in genetic analysis to determine if there has been a loss of genetic variation on Cape Cod over time and to assess how different Cape Cod populations are compared to other hognose snake populations. Additionally, at this time it is not possible to determine if the lack of genetic diversity and the current distribution of private alleles are due to sampling error from small sample size. Future work will focus on increasing sample sizes from throughout the Cape Cod region to determine if low genetic diversity and unique, private alleles are truly representative of *H. platirhinos*.

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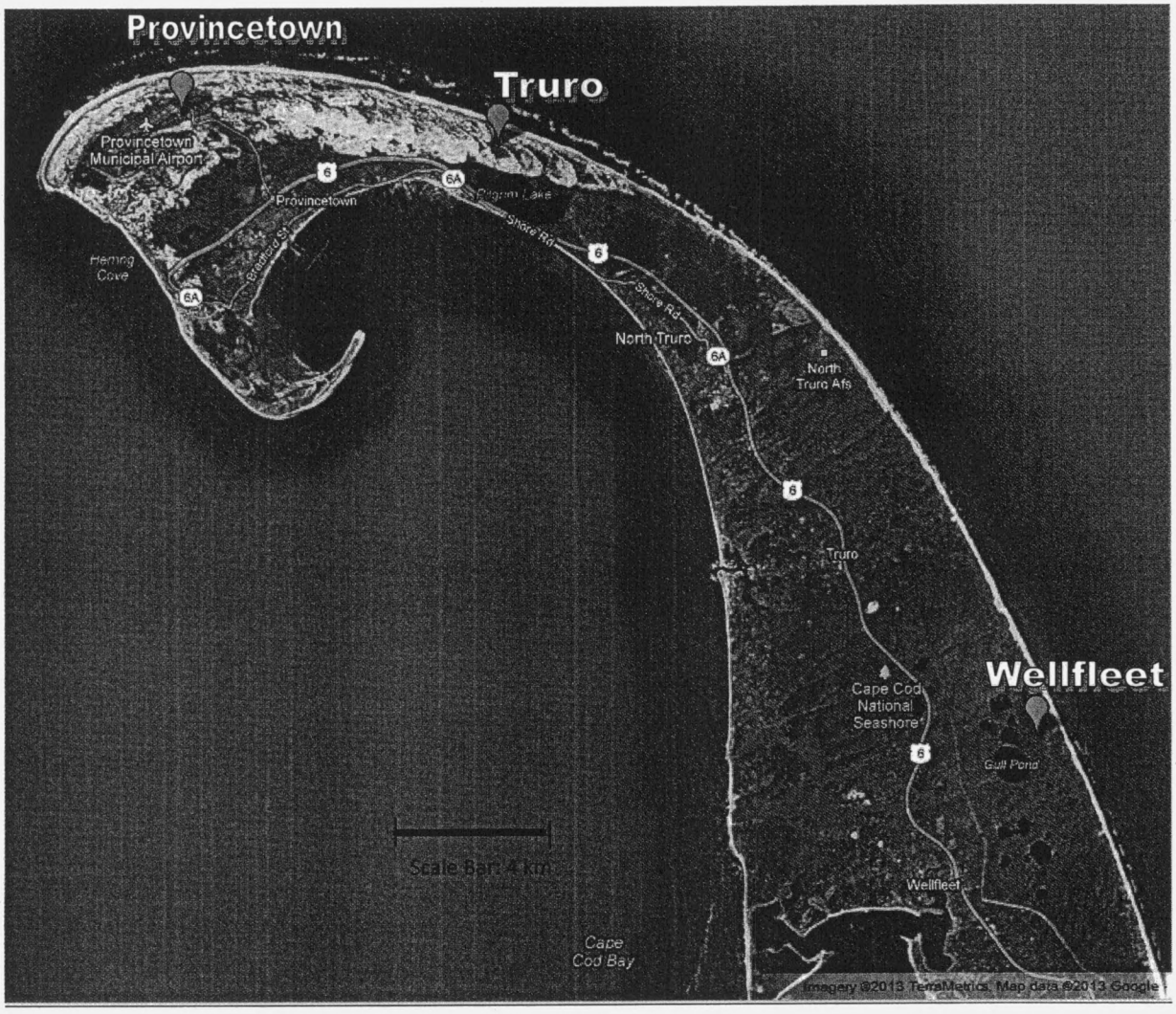
APPENDIX A

MAP OF THE LOCATION OF STUDY SITES

APPENDIX A

Figure 4. Map of location of study sites; Cape Cod, Massachusetts. The northernmost sites, Provincetown and Truro, are separated by approximately six kilometers of an unbroken patch of sand dunes, shrubland, and temporary wetlands. The third study site, Wellfleet, is separated from Truro by approximately sixteen kilometers, most of which is pine forest with very few wetlands.



APPENDIX B

DATA FOR *Heterodon platirhinos* SAMPLES ANALYZED IN THIS STUDY

APPENDIX B

Table 2. Data for *Heterodon platirhinos* samples analyzed in this study. The data for each individual include this study's identification number and source's identification number, the location – area, town, and GPS coordinates - of the location of collection, the type of sample collected, and any notes pertaining to the samples. Abbreviations for the notes are as follows: asterisk (*) indicates a sample with quality sequences which were used in this study. One (1) in the notes column indicates a sequence of low quality or shorter than 151 bp which was not used in data analysis. Two (2) in the notes column indicates a tissue or blood sample for which high quality DNA was not obtained and therefore no sequences were generated.

ID Number	Source ID	Tissue Type	Easting	Northing	Location	Town	Notes
HN 1	S061510	Swab/Scales	416721	4645778	Peck House	Wellfleet	*
HN 3	N83010	Swab/Scales/Fecal	399609	4658143	Province Lands Dunes	Provincetown	*
HN 5	S080209	Scales	417199	4642267	Power Lines	Wellfleet	*
HN 6	N070109	Scales	399722	4658232	Province Lands	Provincetown	*
HN 9	N0901102	Scales/Swab	400454	4658780	PL Visitor Center	Provincetown	*
HN 10	S053010	Swab/Scales/Fecal	416785	4645626	Valley Road	Wellfleet	*
HN 11	N04031103	Scales/Swab	399086	4657574	Province Lands	Provincetown	*
HN 12	N0901101	Scales/Swab	400454	4658780	PL Visitor Center	Provincetown	*
HN 13	N051609	Blood	399183	4657287	Hatches Harbor	Provincetown	*
HN 14	N0815104	Scales/Swab	408291	4657102	High Head	Truro	*
HN 15	N070309	Scales	408355	4657236	High Head	Truro	*
HN 16	S062002	Tail Segment	Unknown	Unknown	Ocean View Dr.	Wellfleet	*
HN 17	N061610	Blood/Swab/Scales	398535	4657296	Hatches Harbor	Provincetown	1
HN 18	N052410	Blood/Swab	400277	4658681	PL Visitor Center	Provincetown	1
HN 19	N081110	Removed Tail	407839	4656998	High Head	Truro	1
HN 20	S062410	Swab/Scales	416772	4645838	Peck House	Wellfleet	*
HN 21	N100810	Swab/Scales/Fecal	339529	4659224	Race Point	Provincetown	1
HN 22	S062510	Swab/Scales	416772	4645838	Peck House	Wellfleet	*
HN 23	N062009	Scales	408276	4657282	High Head	Truro	*
HN 24	N091710	Scales/Swab	399364	4659101	Race Point	Provincetown	*
HN 25	N04031102	Scales/Swab	399086	4657574	Province Lands	Provincetown	*
HN 26	N0510092	Blood	408366	4657319	High Head	Truro	*
HN 27	N080509	Blood	399271	4657316	Race Point	Provincetown	1
HN 28	N052309	Blood	397216	4657754	Race Point	Provincetown	1
HN 29	N052210	Blood/Swab/Scales	398556	4657352	Hatches Harbor	Provincetown	*
HN 30	N0510091	Blood	408284	4657270	High Head	Truro	1
HN 33	60911A	Scales	400300	4658591	PL Visitor Center	Provincetown	*

HN 34	81211A	Swab/Scales/Fecal	399846	4659152	Unknown	Provincetown	*
HN 35	081211B	Swab/Scales/Fecal	398608	4653280	Unknown	Provincetown	*
HN 36	092911A	Swab/Scales/Fecal	398689	4657184	Race Point	Provincetown	*
HN 39	N071409	Scales	398574	4657299	Hatches Harbor	Provincetown	1
HN 40	N04031101	Scales/Swab	408019	4656825	High Head	Truro	2
HN 41	N050109	Blood	398900	4656153	Province Lands Road	Provincetown	2
HN 42	N050510	Blood/Swab	400308	4658630	PL Visitor Center	Provincetown	2
HN 43	N050809	Blood	399199	4657317	Hatches Harbor	Provincetown	2
HN 44	N060209	Blood	399579	4658143	Cranberry Lot	Provincetown	2
HN 45	N060609	Blood	398703	4656984	Hatches Harbor	Provincetown	2
HN 46	N060610	Blood/Swab/Scales	399604	4659266	Race Point	Provincetown	2
HN 47	N061710	Blood/Swab/Scales	399599	4659187	Race Point	Provincetown	2
HN 48	N0627101	Scales/Swab	410517	4656160	Head of the Meadow	Truro	2
HN 49	N0627102	Scales/Swab	400186	4658950	PL Visitor Center	Provincetown	2
HN 50	N072009	Blood	397484	4657931	Race Point	Provincetown	2
HN 51	N072810	Scales/Swab	399600	4659073	Race Point	Provincetown	2
HN 52	N081410	Scales/Swab	408291	4657102	High Head	Truro	2
HN 53	N0815101	Scales/Swab	408291	4657102	High Head	Truro	2
HN 54	N0815102	Scales/Swab	408291	4657102	High Head	Truro	2
HN 55	N0815103	Scales/Swab	408291	4657102	High Head	Truro	2
HN 56	N0815105	Scales/Swab	408291	4657102	High Head	Truro	2
HN 57	N081610	Scales/Swab	408291	4657102	High Head	Truro	2
HN 58	N081810	Scales/Swab	407881	4657113	High Head	Truro	2
HN 59	N090810	Scales	400454	4658780	PL Visitor Center	Provincetown	2
HN 60	N091307	Tail Segment	400454	4658780	PL Visitor Center	Provincetown	2
HN 61	S052009	Scales	416772	4645838	Peck House	Wellfleet	2
HN 62	S071709	Scales	416772	4645838	Peck House	Wellfleet	2