Mitochondrial DNA Sequence Variation in White-Tailed Deer (Odocoileus virginianus) in New England: Evidence for Founder Effect on Nantucket Island

Article *in* Northeastern Naturalist · November 2023

DOI: 10.1656/045.030.0401

CITATIONS
0

READS
58

3 authors:

Richard Beckwitt
Framingham State University
20 PUBLICATIONS **355** CITATIONS
SEE PROFILE

Sarah Treanor Bois
Linda Loring Nature Foundation
20 PUBLICATIONS **265** CITATIONS
SEE PROFILE

Bryan A. Connolly
Eastern Connecticut State University
67 PUBLICATIONS **642** CITATIONS
SEE PROFILE

Mitochondrial DNA Sequence Variation in White-tailed Deer (*Odocoileus virginianus*) in New England: Evidence for Founder Effect on Nantucket Island

Richard Beckwitt[1,*], Sarah Bois[2], and Bryan Connolly[3]

Abstract - *Odocoileus virginianus* (White-tailed Deer) currently number ~3000 on Nantucket Island. No deer were seen on the island at the beginning of the 20th century. The historical record suggests that a single male deer was brought to the island in 1922, and that 2 female deer were brought to the island from Michigan in 1926. After the deer population had increased to several hundred, additional deer (2 male and 3 female) were brought to Nantucket from New Hampshire in 1935 and 1936. To investigate the presence of founder effect in the population on Nantucket, we obtained samples of White-tailed Deer feces or muscle tissue from Nantucket; the New England mainland (including Cape Cod, southeastern Massachusetts, and a few samples from Maine, Connecticut, and Rhode Island), Shelter Island, NY; and Ann Arbor, MI. We amplified a portion of the mitochondrial control region (D-loop), and found 3 different sequences (haplotypes) among 35 deer samples from Nantucket. Two common haplotypes were identical or nearly identical to haplotypes from Michigan. One rare haplotype was also found in deer from the mainland in Connecticut and Massachusetts. This latter haplotype was unusual in that it contained 3 tandem copies of a 75 base-pair repeat, while most White-tailed Deer have 2 copies. In contrast, we found 5 haplotypes among 26 deer from the New England mainland. Haplotype diversity on Nantucket was 0.447 (± 0.082), and nucleotide diversity (π) was 0.021 (± 0.005). Haplotype diversity on the mainland was 0.839 (± 0.029), and π was 0.046 (± 0.002). Analysis of molecular variance (AMOVA) indicated little genetic differentiation among populations on the New England mainland ($\phi_{ST} = 0.095$, $P = 0.113$). However, when the population on Nantucket was included in the analysis, there was much more genetic variation among populations ($\phi_{ST} = 0.414$, $P = 0.000$). Our results indicate that most deer on Nantucket originated from 2 founding females from Michigan, and a small percentage are descended from later introductions from the New England mainland.

Introduction

The current distribution of *Odocoileus virginianus* (Zimmermann) (White-tailed Deer) is the result of biogeography and human intervention (Heffelfinger 2011). At the end of the last Ice Age, deer expanded their range northward as far as Canada, and from the Atlantic Ocean to the Rocky Mountains and beyond. White-tailed Deer have been subdivided into at least 28 subspecies, based on morphology and geography, although many of these subspecies are in dispute. Deer in the northeastern United States have been assigned to the subspecies *O. virginianus*

[1]Department of Biology, Framingham State University, Framingham, MA 01701. [2]Linda Loring Nature Foundation, Nantucket, MA 02554. [3]Department of Biology, Eastern Connecticut State University, Willimantic, CT 06226. [*]Corresponding author - rbeckwitt@framingham.edu.

Manuscript Editor: James Cardoza

borealis Miller (Northern White-tailed Deer; Heffelfinger 2011). After European colonization of North America, White-tailed Deer populations declined severely in many parts of the United States. More recently, many regions have been repopulated using animals translocated from other (often distant) regions, making regional differentiation more obscure (Chafin et al. 2021).

The population genetics of White-tailed Deer and the closely related *Odocoileus hemionus* (Rafinesque) (Mule Deer) have been well-studied in several regions of North America (Anderson et al. 2002, Bauder et al. 2021, Cullingham et al. 2011, DeYoung et al. 2003, Latch et al. 2009, Purdue et al. 2006). Mitochondrial DNA (mtDNA) in mammals is maternally inherited (Sato and Sato 2013), passed down from mother to offspring with no male contribution. Mitochondrial sequence variations have been widely used to examine the population and subspecies structure of White-tailed Deer, as well as to estimate the risk of the spread of chronic wasting disease (Budd et al. 2018). Mitochondrial DNA has also been used to detect evidence of recent population expansions in a wide variety of organisms (Fu 1997, Ramos-Onsins and Rozas 2002). Latch et al. (2009) found evidence for a recent population expansion in Mule Deer, but a similar study failed to find such evidence in White-tailed Deer in Missouri (Budd et al. 2018).

The deer population on Nantucket Island, MA, is one of the densest in the Northeast, estimated to be at ~3000 animals (12–20/km^2; MassWildlife n.d.). The history of White-tailed Deer on Nantucket is not entirely clear. Deer remains have been found in pre-historic Wampanoag sites on the island (Waters 1965), but historical records suggest that there were no deer on the island at the start of the 20th century (Morral and White 2015). Newspaper accounts record the rescue of a single male White-tailed Deer, swimming in Nantucket Sound, in 1922 (Deer brought to …1922). This single individual was repeatedly seen for several years. Two female White-tailed Deer, purchased from a deer farm in Michigan, were brought to Nantucket in 1926 (Deer liberated in … 1926). It is widely believed on Nantucket that all of the deer on the island are descended from these 3 individuals (Morral and White 2015). From this initial introduction, the herd rapidly grew to about 300 deer by 1935. However, later newspaper reports document the addition of 2 male White-tailed Deer in 1935 and 3 female White-tailed Deer in 1936, all from New Hampshire (Two deer liberated 1935, Three doe liberated 1936).

If the account of the origins of the deer on Nantucket is correct, this successful population was founded initially by only 3 individuals, which would be an extreme example of the founder effect (Wright 1942). If the hypothesis that there were only 2 founding females for the Nantucket population is true, then there should only be 2 distinct mtDNA sequences (haplotypes) found in most of the deer on the island. In addition, mtDNA sequences from Nantucket should more closely resemble those of the midwestern US than those found on the mainland of New England. Mitochondrial haplotypes more typical of the New England region should be rare on Nantucket, since mainland deer were only added to the herd after it had grown to several hundred. The alternate hypothesis, that Nantucket was populated by deer from the adjacent mainland, would predict that Nantucket deer should more closely resemble deer from the Cape Cod region of Massachusetts.

There have been no published studies of the population genetics of deer from New England. Here we report on the mtDNA sequence variation in deer from Nantucket as compared to sequence data for deer in Michigan as well as other parts of New England. Our goal was to test the hypothesis that deer on Nantucket have a genetic history distinct from other deer in the region and indicative of the founder effect, as suggested by the historical account of the population's origin.

Methods

Sampling

We collected 13 fecal samples from various sites on Nantucket Island, MA, during July–August, 2016. Although some effort was made to collect only fresh-appearing fecal samples, we could not accurately estimate their age. After collection, we stored the fecal samples at 4 °C in tissue-preservation buffer (TPB: 20% DMSO, 0.25 M EDTA, saturated with NaCl, pH 8.0; Seutin et al. 1991). We obtained fresh muscle-tissue samples (~1 g each) from Massachusetts Division of Fisheries and Wildlife (DFW) hunter check stations on Nantucket, MA (26 samples), Cape Cod, MA (8 samples), and southeastern Massachusetts (Bristol, Norfolk and Plymouth counties, 14 samples) and preserved all samples at 4 °C in TPB. Samples from Ann Arbor, MI (6), preserved in TPB, were donated by A. Reznieck, University of Michigan, Ann Arbor, MI. Frozen muscle samples from Shelter Island, NY (6), Rangeley, ME (1), Mansfield, CT (2), and Scituate, RI (1), were donated by local hunters and stored at -20 °C.

Genetic analysis

DNA extraction, PCR, and sequencing. We extracted total DNA from single fecal pellets using the Bioline Isolate Fecal DNA kit (Bioline, London, UK) and from 50–100 mg muscle tissue using the Bioline Isolate II Genomic DNA kit. We performed polymerase chain reaction (PCR) using the Platinum™ II Hot-Start PCR Master Mix (Invitrogen, Carlsbad,CA). We amplified a portion of the mtDNA control region corresponding to bases 10407–11106 of the complete White-tailed Deer mtDNA genome, GenBank ID: NC015247.1 (Seabury et al. 2011) using the primers and amplification protocol from Cullingham et al. (2011)—forward primer: 5'-TCTCCCTAAGACTCAAGGAAG-3', reverse primer: 5'-GTCATTAGTCCATCGAGATGTC-3'. PCR consisted of a denaturation step of 94 °C for 5 min, followed by 30 cycles of amplification at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s, with a final extension of 2 min at 72 °C. We amplified again the samples that produced PCR products of 2 different sizes using a touchdown PCR procedure (Don et al. 1991, Roux 1994) to avoid the amplification of double PCR products. With this procedure, the annealing temperature is initially set quite high (64 °C) and lowered by 1 °C decrements until the final annealing temperature of 54 °C is reached. We purified PCR products using the Bioline Isolate II PCR and Gel kit. We then combined the purified PCR products with the forward or reverse PCR primer and sent them to the Molecular Biology Core Labs, University of Massachusetts Medical School, Worcester, MA, for sequencing.

Statistical methods. We edited and aligned sequences using MEGA11 (Tamura et al. 2021). We calculated basic population genetics statistics, haplotype diversity and nucleotide diversity measures, using DnaSP v. 6.12 (Rozas et al. 2017). The haplotype diversity measure (Hd) is based on the number and frequency of haplotypes found in a population, while nucleotide diversity (π) compares the average number of nucleotide differences per site between 2 DNA sequences in all possible pairs in the population (Nei 1987). We also calculated neutrality tests for recent population expansion using DnaSP. To estimate the amount of genetic differentiation among populations, we performed analysis of molecular variance (AMOVA) using Arlequin 3.5 (Excoffier and Lischer 2010). To show the degree of sequence differences among haplotypes, we computed and drew a parsimony-based haplotype network (TCS network; Templeton et al. 1992) using PopArt (Leigh and Bryant 2015). Using MEGA11, we computed and drew a maximum parsimony tree using to illustrate evolutionary relationships and a neighbor-joining tree to show genetic distance relationships. In sequence analyses, we treated the presence or absence of the third 75-bp repeat as a single character trait.

Results

Sequencing

We obtained mtDNA sequences from 73 White-tailed Deer samples. Only 9 of 13 fecal samples produced useable DNA sequences. All muscle samples (fresh or frozen) produced useable sequences. The expected size of the PCR product was 699 base pairs (bp). Although most PCR products were of the expected size, several samples produced PCR products that were distinctly larger. After editing and alignment, we identified 13 haplotypes (Table 1). Most of the haplotypes were between 590 and 596 bp, and differed by single-base substitutions and single-base insertions/deletions. One haplotype (h3) was 75 bp longer. Most White-tailed Deer mtDNA contain 2 tandem repeats of 75 bp within the part of the control region (D-loop) that we amplified, although some contain 3 or 4 (Purdue et al. 2006). Our

Table 1. MtDNA haplotypes from White-tailed Deer.

Haplotype	Location first observed	Length (bp)	GenBank accession #
h1	Nantucket, MA	591	MF314463
h2	Nantucket, MA	590	MF314462
h3	Nantucket, MA	666	MF314461
h4	Ann Arbor, MI	592	OQ434670
h5	Ann Arbor, MI	591	OQ434671
h6	Shelter Island, NY	591	OQ434672
h7	Ann Arbor, MI	592	OQ434673
h8	Cape Cod, MA	590	OQ434674
h9	Cape Cod, MA	596	OQ434675
h10	Cape Cod, MA	592	OQ434676
h11	Cape Cod, MA	593	OQ434677
h12	Shelter Island, NY	591	OQ434678
h13	Shelter Island, NY	591	OQ434679

shorter PCR products all contained 2 repeats, while h3 contained 3. Haplotypes h3 and h8 are identical except for the presence of the third repeat in h3. On a maximum-parsimony tree, our haplotypes fall into 3 haplogroups (Fig. 1). Haplogroup A contains haplotypes h1, h2, h4, h5, h6, h12, and h13. Haplogroup B contains haplotypes h3 and h8. Haplogroup C contains haplotypes h7, h9, h10, and h11. A haplotype network (TCS network; Templeton et al. 1992) shows the minimum number of mutations among haplotypes as well as their locations (Fig. 2).

Within-population diversity

To test whether deer from Nantucket had reduced genetic diversity, we treated all samples from Nantucket as 1 population, from Cape Cod as a second population, and from the remainder of Massachusetts, along with a few deer from Maine, Connecticut, and Rhode Island, as a third population (New England). We considered samples from Shelter Island, NY, and from Ann Arbor, MI, to be separate populations. Measures of genetic diversity varied considerably among the different populations (Table 2). Both Hd and π are substantially lower on Nantucket than any of the mainland populations (Hd = 0.447 [± 0.082], π = 0.021 [± 0.005]). Haplotype h3 was only found in 2 fecal samples on Nantucket, separated by about 100 m. The possibility exists that both samples are from the same deer. A reanalysis of the data from Nantucket, including only a single h3 sequence, reduced measures of genetic diversity slightly from that shown in Table 2 (Hd = 0.415 [± 0.081], and π = 0.017 [± 0.004]).

Population differentiation

To test whether the population on Nantucket is distinct from other parts of New England, we generated a neighbor-joining tree of average genetic distances among

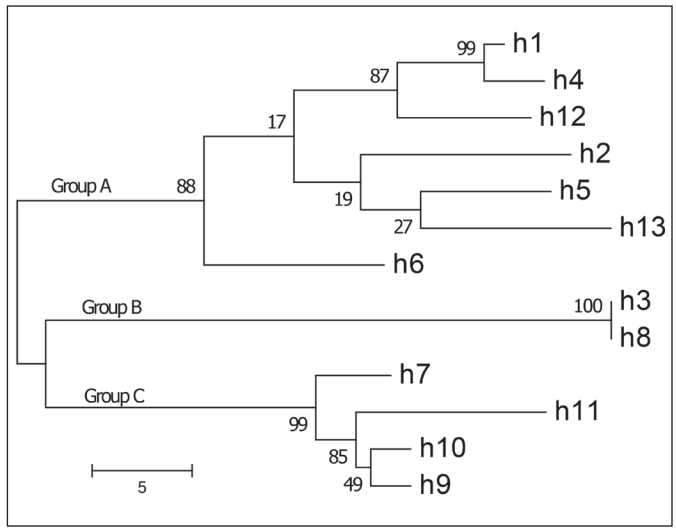

Figure 1. Maximum parsimony tree of haplotypes from this study. A single instance of each haplotype was used to construct the tree. Sites with gaps or missing data were excluded. The percentage of replicate trees in which the associated haplotypes clustered together in the bootstrap test (500 replicates) are shown next to the branches. The total length is 139 steps; the scale bar is in units of the number of changes over the whole sequence. Note: haplotypes h3 and h8 differ only in the presence/absence of a third 75-bp repeat.

populations (Fig. 3). In this tree, the Nantucket population grouped with Michigan rather than other parts of New England. The most-frequent haplotypes on Nantucket (h1 and h2) are not found in any other samples from New England, although haplotype h2 is found in Michigan. Haplotype h1 is also very similar to haplotype h4 from Michigan (587/592 bases identical). Haplotype h3 is rare on Nantucket, but fairly widespread on the mainland of New England.

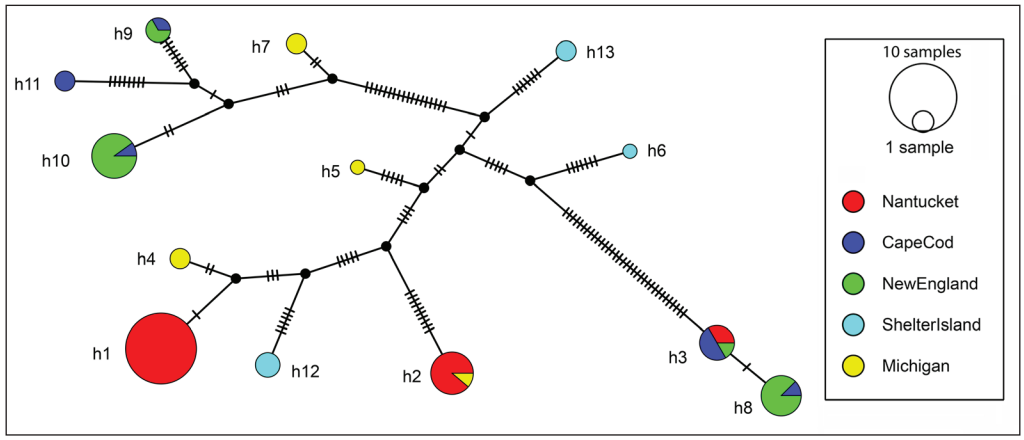

Figure 2. TCS network of haplotypes. Hashmarks indicate the number of mutations between haplotypes. The size of a circle is proportional to the number of samples with that haplotype. Colors indicate populations; see Table 2 for an explanation of population names.

Table 2. Genetic diversity measures of White-tailed Deer. New England includes samples from MA (excluding Nantucket and Cape Cod), ME, CT and RI.

Population	Sample size	Haplotypes	Haplotype diversity (Hd ± SD)	Nucleotide diversity (π ± SD)
Nantucket, MA	35	h1, h2, h3	0.447 (± 0.082)	0.021 (± 0.005)
Cape Cod, MA	8	h3, h8, h9, h10, h11	0.857 (± 0.108)	0.049 (± 0.008)
New England	18	h3, h8, h9, h10	0.627 (±0.073)	0.041 (± 0.004)
Ann Arbor, MI	6	h2, h4, h5, h7	0.867 (± 0.129)	0.044 (± 0.007)
Shelter Island, NY	6	h6, h12, h13	0.733 (± 0.155)	0.030 (± 0.006)

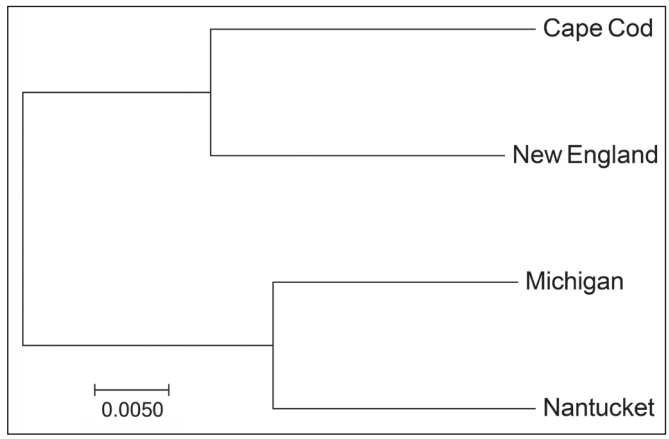

Figure 3. Neighbor-joining tree of mean group distances for White-tailed Deer populations. The population on Nantucket is more similar to a sample from Michigan than it is to other locations in New England. See Table 2 for explanation of population names. The scale is in units of p-distance (fraction of sites that differ between sequences).

We estimated the amount of genetic differentiation among populations of deer using AMOVA (Table 3). AMOVA calculates the total genetic diversity (the frequency of different haplotypes as well as how similar the sequences are) and estimates how much of the diversity is due to differences among individuals within each population and how much is due to differences among populations. When the deer of Cape Cod are compared with deer from the remainder of New England, only 9.49% of the genetic diversity is due to differences among populations (ϕ_{ST} = 0.095, P = 0.121). However, when deer from Nantucket are compared to deer from Cape Cod, 49.20% of the genetic diversity is due to differences among populations (ϕ_{ST} = 0.492, P = <0.001). When deer from Nantucket are compared to deer from all parts of the New England mainland, 41.37% of the genetic diversity is due to differences among populations (ϕ_{ST} = 0.414, P = <0.001). When deer from Nantucket are compared to deer from Michigan, 13.73% of the variation is due to differences among populations (ϕ_{ST} = 0.137, P = 0.029). In this case, although the sample from Michigan contains 2 haplotypes that are identical or similar to Nantucket haplotypes (h2 and h4), the other 2 Michigan haplotypes are quite different. The population on Shelter Island, NY, is distinct from all of the other samples and has a confused history of possible introductions from distant sources (Robey 2020). The haplotypes found there (h6, h12, h13) are not found anywhere else in the samples from the Northeast. When Shelter Island is compared to all of the deer on the New England mainland, 31.76% of the variation is due to differences among populations (ϕ_{ST} = 0.318, P = 0.007).

We evaluated evidence for a recent population expansion using both Fu's Fs (Fu 1997) and Ramos-Onsins and Rosas' R2 (Ramos-Onsins and Rozas 2002). Fs is large and negative when the distribution of haplotypes is significantly different

Table 3. AMOVA among populations of White-tailed Deer. See Table 2 for explanation of population names. Probabilities estimated by 10,000 permutations.

Source of variation	df	% variation	Fixation index (ϕ)	P-value
Cape Cod, MA, versus New England				
Among populations	1	9.49	ϕ_{ST} = 0.095	0.113
Within populations	24	90.51		
Nantucket, MA, versus Cape Cod, MA				
Among populations	1	49.20	ϕ_{ST} = 0.492	<0.001
Within populations	41	50.80		
Nantucket, MA, versus New England and Cape Cod, MA, treated as a single population				
Among populations	1	41.37	ϕ_{ST} = 0.414	<0.001
Within populations	59	58.63		
Nantucket, MA, versus Ann Arbor, MI				
Among populations	1	13.73	ϕ_{ST} = 0.137	0.029
Within populations	39	86.27		
Shelter Island, NY, versus New England and Cape Cod, MA, treated as a single population				
Among populations	1	31.76	ϕ_{ST} = 0.318	0.007
Within populations	30	68.24		

from the expectation of stable population size and no selection. When deer from all of New England (excluding Nantucket) were evaluated, both measures were negligible (Fs = -0.127, 95% CI = -5.84–5.98; R2 = 0.120, P = 1.00). The population on Nantucket was similar (Fs = 0.348, 95% CI = -3.54–4.89; R2 = 0.162, P = 1.00).

Discussion

Mitochondrial sequence diversity of deer on Nantucket was low compared to the New England mainland, while the mtDNA diversity of deer from the mainland was similar to other large, outbred populations (Almond 2018, Cullingham et al. 2011). White-tailed Deer have been introduced to several other locations, including the US Virgin Islands (Nelson et al. 2021) and Finland (Brommer et al. 2015). These introduced populations also show reduced genetic diversity. Several studies that used AMOVA have found the pattern seen in New England of very little genetic variation partitioned among populations in other areas: Mississippi (Youngmann 2018), West Virginia (Wood 2018), and Iowa (Almond 2018).

If the popular account were true, that all deer on Nantucket were descended from 1 male and 2 females brought to the island in the 1920s, then one would expect at most 2 distinct mtDNA haplotypes in island deer. Indeed, most of the deer sampled from Nantucket had 1 of 2 common haplotypes. If a few additional deer were brought to the island from New Hampshire in the 1930s, after the population had reached several hundred, then haplotypes of those female deer would be expected to contribute only a small fraction of the mtDNA genetic variation on the island. The presence of 1 or 2 deer samples with haplotype h3, also found throughout New England, is consistent with this scenario. More recent introductions of female deer to Nantucket, after the population had grown to its current size, would contribute only very rare haplotypes that would be difficult to detect without very large sample sizes. A power analysis of the likelihood of finding other rare haplotypes on Nantucket depends on the expected frequency of the rare haplotype. If the true frequency of a haplotype is 0.1, the probability of not finding it in a sample of 35 is P = 0.023. If the true frequency is smaller, than the probability of not finding it increases (with a true frequency = 0.01, probability of not finding it in a sample of 300 is P = 0.049).

Both male and female White-tailed Deer can disperse long distances, although male dispersal distance is usually greater (Lutz et al. 2015). Deer are also strong swimmers, and can occasionally swim to nearby islands (Heffelfinger 2011). However, deer do not seem to be able to swim the 11-km Moser Strait between the upper and lower Florida Keys, since *Odocoileus virginianus clavium* Barbour & G.M. Allen (Key Deer) are a distinct subspecies (Villanova et al. 2017). For comparison, the shortest distance from Cape Cod (Monomoy peninsula) to Nantucket is 20 km, and the shortest distance from Nantucket (Muskeget Island) to Martha's Vineyard (Chappaquiddick Island) is 13 km. If any male deer did swim to Nantucket, they would not be detectable in a survey of mtDNA. It is highly unlikely that female deer frequently swim to Nantucket, since common haplotypes present on Cape Cod are absent from the island. The historical record suggests that deer were completely

absent from Nantucket for a substantial period, and when a single deer was brought to the island, it was repeatedly seen, which also supports the idea that natural dispersal to Nantucket must have been extremely rare.

Our analysis of mtDNA variation in White-tailed Deer from Nantucket agrees with the account that deer on Nantucket are the result of introduction. The population shows clear evidence of founder effect, with likely origins from deer in Michigan. Deer from the New England mainland have genetic diversity similar to other large, outbred populations in North America, and there is little evidence for genetic differentiation among populations within the region, with the exception of Nantucket.

Acknowledgments

Funding for this project was provided by the Framingham State University (FSU) Center for Excellence in Learning, Teaching, Scholarship and Service, and the FSU Biology Department for support of undergraduate research. Much of the work, including preparing samples for DNA sequencing and preliminary sequence editing and analysis, was done by FSU students as part of their undergraduate research projects. The students who contributed to this work include: Natalie C. Buck, Julie T. Banda, Evan Boulet, Katherine J. Downing, Perin E. Genaway, Simran Kaur, Deidra L. Kilgore, Katheryn C. Kinsman, Da Da Le, Christopher Jean Louis, Ava C. Melchin, Rachel E. Mullen, Tina Nguyen, Samael O'Brien, Grace R. Pushor, Autumn Reis, Nunzio Rosselli, Sara Sandes, Robert W. Shea, Amanda E. Stackpole, Upsana Tanvir, Cheryl A. Thomas, Kyle W. Tibbert, Elizabeth C. Wieczorek, Angela Wyman, Sarosh A. Zahra, and Jessica M. Zamboni. Carl Bois donated samples from Nantucket, MA; Thomas Rawinski donated samples from Shelter Island, NY, Rangeley, ME, and Scituate, RI; Anton Reznieck donated samples from Ann Arbor, MI. Seth Engelbourg, Linda Loring Nature Foundation, provided help with sample collection. Steven Wright and Connor Fleming, Massachusetts Division of Fisheries and Wildlife, collected samples from the DFW hunter check stations. This paper was substantially improved by the comments of the manuscript editor and 2 anonymous reviewers.

Literature Cited

Almond, L.C.G. 2018. Using population genetics to examine White-tailed Deer (*Odocoileus virginianus*) connectivity across multiple spatial scales: Influences of proximity, landscape, and history. PhD. Iowa State University. 180 pp. Available online at https://www.proquest.com/openview/acdaaaff72d16457f6e48d0dde5d58f9/1?pq-origsite=gscholar&cbl=18750. Accessed 17 January 2023.

Anderson, J.D., R.L. Honeycutt, R.A. Gonzales, K.L. Gee, L.C. Skow, R.L. Gallagher, D.A. Honeycutt, and R.W. DeYoung. 2002. Development of microsatellite DNA markers for the automated genetic characterization of White-tailed Deer populations. Journal of Wildlife Management 66:67–74.

Bauder, J.M., C.S. Anderson, H.L. Gibbs, M.J. Tonkovich, and W.D. Walter. 2021. Landscape features fail to explain spatial genetic structure in White-tailed Deer across Ohio, USA. Journal of Wildlife Management 85:1669–1684.

Brommer, J.E., J. Kekkonen, and M. Wikström. 2015. Using heterozygosity–fitness correlations to study inbreeding depression in an isolated population of White-tailed Deer founded by few individuals. Ecology and Evolution 5:357–367.

Budd, K., L.K. Berkman, M. Anderson, J. Koppelman, and L.S. Eggert. 2018. Genetic structure and recovery of White-tailed Deer in Missouri. Journal of Wildlife Management 82:1598–1607.

Chafin, T.K., Z.D. Zbinden, M.R. Douglas, B.T. Martin, C.R. Middaugh, M.C. Gray, J.R. Ballard, and M.E. Douglas. 2021. Spatial population genetics in heavily managed species: Separating patterns of historical translocation from contemporary gene flow in White-tailed Deer. Evolutionary Applications 14:1673–1689.

Cullingham, C.I., E.H. Merrill, M.J. Pybus, T.K. Bollinger, G.A. Wilson, and D.W. Coltman. 2011. Broad and fine-scale genetic analysis of White-tailed Deer populations: Estimating the relative risk of chronic wasting disease spread. Evolutionary Applications 4:116–131.

Deer brought to Nantucket. 1922.The Inquirer and Mirror, Nantucket, MA, 10 June 1922, p. 6. Available online at https://digital.olivesoftware.com/olive/apa/nantucket/default.aspx#panel=search&search=1. Accessed 8 February 2023.

Deer liberated in swamps near Squam. 1926. The Inquirer and Mirror, Nantucket, MA, 23 January 1926, p. 4. Available online at https://digital.olivesoftware.com/olive/apa/nantucket/default.aspx#panel=search&search=1. Accessed 8 February 2023.

DeYoung, R.W., S. Demarais, R.L. Honeycutt, A.P. Rooney, R.A. Gonzales, and K.L. Gee. 2003. Genetic consequences of White-tailed Deer (*Odocoileus virginianus*) restoration in Mississippi. Molecular Ecology 12:3237–3252.

Don, R.H., P.T. Cox, B.J. Wainwright, K. Baker, and J.S. Mattick. 1991. "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research 19:4008.

Excoffier, L., and H.E.L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925.

Heffelfinger, J.R. 2011. Taxonomy, evolutionary history, and distribution. Pp. 3–39, *In* D.G. Hewitt (Ed.). Biology and Management of White-tailed Deer. CRC Press, Baton Rouge, LA. 686 pp.

Latch, E.K., J.R. Heffelfinger, J.A. Fike, and O.E. Rhodes Jr. 2009. Species-wide phylogeography of North American Mule Deer (*Odocoileus hemionus*): Cryptic glacial refugia and postglacial recolonization. Molecular Ecology 18:1730–1745.

Leigh, J.W., and D. Bryant. 2015. PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116.

Lutz, C.L., D.R. Diefenbach, and C.S. Rosenberry. 2015. Population density influences dispersal in female White-tailed Deer. Journal of Mammalogy 96:494–501.

MassWildlife. n.d. Deer management. Available online at https://www.mass.gov/service-details/deer-management. Accessed 21 February 2023.

Morral, F., and B.A. White. 2015. Hidden History of Nantucket. The History Press, Charleston, SC. 128 pp.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY. 512 pp.

Nelson, S.L., S.A. Taylor, and J.D. Reuter. 2021. An isolated White-tailed Deer (*Odocoileus virginianus*) population on St. John, US Virgin Islands, shows low inbreeding and comparable heterozygosity to other larger populations. Ecology and Evolution 11:2775–2781.

Purdue, J.R., T.K. Oleksyk, and M.H. Smith. 2006. Independent occurrences of multiple repeats in the control region of mitochondrial DNA of White-tailed Deer. Journal of Heredity 97:235–243.

Ramos-Onsins, S.E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

Robey, C. 2020. Deer, and the island's attempts to control them. Shelter Island Reporter. 21 October 2020. Available online at https://shelterislandreporter.timesreview.com/2020/10/21/deer-and-the-islands-attempts-to-control-them/. Accessed 16 February 2023.

Roux, K.H. 1994. Using mismatched primer-template pairs in touchdown PCR. BioTechniques 16:812–814.

Rozas, J., A. Ferrer-Mata, J.C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S.E. Ramos-Onsins, and A. Sánchez-Gracia. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34:3299–3302.

Sato, M., and K. Sato. 2013. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. Biochimica et Biophysica Acta 1833:1979–1984.

Seabury, C.M., E.K. Bhattarai, J.F. Taylor, G.G. Viswanathan, S.M. Cooper, D.S. Davis, S.E. Dowd, M.L. Lockwood, and P.M. Seabury. 2011. Genome-wide polymorphism and comparative analyses in the White-tailed Deer (*Odocoileus virginianus*): A model for conservation genomics. PLOS ONE 6:e15811.

Seutin, G., B.N. White, and P.T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. Canadian Journal of Zoology 69:82–90.

Tamura, K., G. Stecher, and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38:3022–3027.

Templeton, A.R., K.A. Crandall, and C.F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

Three doe liberated. 1936. The Inquirer and Mirror, Nantucket, MA, 18 April 1936, p. 4. Available online at https://digital.olivesoftware.com/olive/apa/nantucket/default.aspx#panel=search&search=1. Accessed 8 February 2023.

Two deer liberated. 1935. The Inquirer and Mirror, Nantucket, MA, 20 April 1935, p. 4. Available online at https://digital.olivesoftware.com/olive/apa/nantucket/default.aspx#panel=search&search=1. Accessed 8 February 2023.

Villanova, V.L., P.T. Hughes, and E.A. Hoffman. 2017. Combining genetic structure and demographic analyses to estimate persistence in endangered Key Deer (*Odocoileus virginianus clavium*). Conservation Genetics 18:1061–1076.

Waters, J.H. 1965. Animal remains from some New England sites. Bulletin of the Archaeological Society of Connecticut 33:5–12. Available online at http://hdl.handle.net/11134/20002:ASCBulletin. Accessed 10 July 2023.

Wood, D. 2018. The use of genetic tools to assist in White-tailed Deer (*Odocoileus virginianus*) management in West Virginia. PhD. West Virginia University. 157 pp. Available online at https://researchrepository.wvu.edu/etd/8213. Accessed 26 January 2023.

Wright, S. 1942. Statistical genetics and evolution. Bulletin of the American Mathematical Society 48:223–246.

Youngmann, J.L. 2018. Genetic assessment of native and non-native White-tailed Deer (*Odocoileus virginianus*) in the southcentral US. M.Sc. Thesis. Mississippi State University, , MS. 94 pp. Available online at https://scholarsjunction.msstate.edu/td/2394/. Accessed 10 February 2023.