

*An Analysis of Genetic Diversity in the Pennsylvania
State Population of White-Tailed Deer (Odocoileus
virginianus) 110 Years After Bottleneck and
Translocation*

By
Michael Richard Arcieri
April 3, 2017



Allegheny College
Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College

**I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole.**

Pledge

Approval by the Senior Thesis Committee:

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Abstract

In wildlife conservation, genetic rescue is an attempt to combat the negative genetic effects of population bottleneck by increasing genetic diversity through translocation of new individuals into a threatened population. This method has been used extensively to preserve genetic diversity in overhunted populations, but concerns exist over the long-term consequences of these conservation efforts. White-tailed deer (*Odocoileus virginianus*) in the United States suggest long-term translocation success, with present population density at its highest point since European settlement and genetic diversity comparable to deer herds untouched by European hunting. Deer in Pennsylvania represent one of the oldest examples of these translocations, supporting the method as a viable means of genetic rescue. However, no current studies have analyzed the genetic diversity of this population. We used microsatellite analysis to assess genetic diversity at two loci across a sample of Pennsylvania white-tailed deer collected from hunters, testing our results for genetic bottleneck and comparing them to other recovered white-tailed deer populations. Our results suggest that Pennsylvania deer exhibit no evidence of genetic bottleneck and a level of genetic diversity comparable to recovered deer populations, supporting translocation as a sustainable means to maintain genetic diversity when increasing the size of a threatened population.

Introduction

The International Union for Conservation of Nature and Natural Resources (IUCN) estimates that 30% of all known species are endangered globally, labeling the current extinction epidemic a crisis. Human activity is responsible for nearly 85% of these endangerments (IUCN Biodiversity Conservation Group 2015). More specifically, the unsustainable hunting of species (overhunting) has played a key role in reducing global biodiversity. For example, in a survey of 232 separate oceanic communities of commercial fish, population size has declined by an average of 83% since 1980 as a direct result of overfishing (Hutchings and Reynolds 2004). Large aquatic carnivores such as sharks, which are crucial for maintaining biodiversity in oceanic food webs, are directly affected by overfishing when they are caught as bycatch. Removing sharks' prey from oceanic habitats also indirectly contributes to their decline. In the Northwest Atlantic, a region dominated by global fisheries, nearly all shark species have experienced a 50% decline in population size since 1980 (Baum et al. 2003). Furthermore, local extinctions have occurred in populations of sea otter, northern elephant seal, dugong, five species of skate, and three species of sharks, although no recorded species have experienced global extinction from overfishing (Dulvy et al. 2003). On land, 22% of mammalian species are listed on the IUCN Red List, a list comprised of vulnerable, endangered, or critically endangered species. An analysis of this Red List determined that between 2000 and 2010, 62 species of mammals moved closer to extinction as a result of unregulated, subsistence hunting (Hoffmann et al. 2010). For example, 41 "bush meat" species (i.e., deer) have experienced regional decline in West Africa over the past 30 years (Brashares et al. 2004).

Some debate exists as to whether commercial overhunting could lead to global extinctions (Beverton 1990; Dulvy et al. 2003). Yet, it is well established that overhunting can severely impact conservation efforts by creating severe population bottlenecks (Chabot et al. 2015). Population bottlenecks, defined as a dramatic reduction in population size over a short span of generations, can result in inbreeding and genetic drift. In small populations, related individuals may breed, reducing fitness of the population through a phenomenon called inbreeding depression (Frankham 1996). In the lab, inbreeding depression has been documented in fruit flies, where resistance to bacterial toxin and fungal infection was significantly lower in small, inbreeding groups than in large, randomly mating groups (Spielman et al. 2004). Similar results have been observed in the wild. Lower levels of genome-wide heterozygosity in wild harbor seals predicted higher susceptibility to parasite infection in individuals (Hoffman et al. 2014). In little spotted kiwis, hatching success was significantly lower in a bottlenecked population than in the source population, despite the bottlenecked population making a full recovery in terms of population size (Taylor et al. 2017).

Genetic drift occurs when random changes in allele frequencies become amplified by small population sizes, causing alleles to go to fixation or be lost (Frankham 1996). Genetic drift is evident in the extreme bottleneck of Guadalupe Fur Seals, which were recovered from a population of just 17 seals to a population of over 12,000 individuals through conservation efforts. Prior to the bottleneck event, 25 mitochondrial haplotypes were present in the population, but only 7 haplotypes are now found in the recovered population (Weber et al. 2004). Both inbreeding depression and genetic drift decrease heterozygosity, allelic richness, and overall genetic variation within a population. These genetic consequences directly

decrease the fitness of individuals in the population, affecting longevity and reproductive success (Brommer et al. 2015; Taylor et al. 2017). In a meta-analysis of 34 populations across a variety of taxa, the level of genetic diversity within a population was found to have a moderate positive correlation ($r=0.432$) with the population's fitness (Reed and Frankham 2003).

To combat the effects of inbreeding depression and genetic drift, conservation efforts for bottlenecked populations have focused on increasing genetic diversity in the population in addition to increasing the population size itself, an idea known as genetic rescue (Frankham 2015). Translocation, the introduction of new organisms into an isolated population, has become a popular method for restoring genetic diversity and reducing the effects of inbreeding depression in threatened populations. Yet, no clear consensus exists on the long-term effects of translocation as a means of improving genetic diversity. Gene flow into an isolated population has been shown to reduce inbreeding depression and genetic drift in recently recovered populations of otters, adders, and wolves (Arrendal et al. 2004; Bouzat et al. 2009; Madsen et al. 1999; Vilà et al. 2003). However, translocations may also hinder long-term fitness of isolated populations by introducing individuals with maladaptive traits into the isolated population's habitat, decreasing population fitness through a phenomenon known as outbreeding depression (Edmands 2007). This concern has been documented in intertidal copepods, where hatching numbers, survival, and morphometric fitness significantly decreased after two genetically isolated populations were bred together (Hwang et al. 2012). Furthermore, since translocations are often used as a last resort in wildlife conservation, most of them are conducted without prior genetic evaluation of the threatened

or source populations, meaning genetic implications are not fully considered in these management decisions (Arrendal et al. 2004).

One example of long-term translocation success is the resurgence of white-tailed deer (*Odocoileus virginianus*) in the United States. Prior to 1900, unregulated hunting by European settlers led to widespread decline of white-tailed deer across America. By 1900, population sizes had been reduced to <1000 individuals per state (Figure 1) (Blackard 1971; Gassett 2001). Between 1930 and 1965, the Mississippi Department of Wildlife, Fisheries, and Parks translocated 3,208 deer into the state from Alabama, Kentucky, Louisiana, Mexico, North Carolina, and Wisconsin. From 1946 to 1952, the newly created Kentucky state wildlife refuge also began translocating deer into Kentucky from the same donor state populations used in the Mississippi translocations (Blackard 1971; Gassett 2001). Today, Mississippi has the highest population density of white-tailed deer in the United States, with a total population of 1.75 million deer statewide (MDWFP Deer Committee 2012). Kentucky has also rebounded to an estimated population of 752,089 deer statewide, based on harvest and age structure data from 2012 (Kentucky Department of Fish and Wildlife Resources 2013). In fact, white-tailed deer populations have rebounded past pre-settlement numbers, attributed to the extirpation of their natural predators via hunting (Blackard 1971; VerCauteren 2003). Translocation success has been supported by a recent genetic analysis which revealed high gene diversity and observed heterozygosity across microsatellite DNA markers in a sample of deer from Mississippi (21 loci) and Kentucky (7 loci). Furthermore, the level of genetic diversity found in Kentucky deer is comparable to the level found in New England white-tailed deer, a population which never experienced overhunting and is believed

to be representative of the genetic diversity found within the North American deer population prior to European settlement (Doerner et al. 2005).

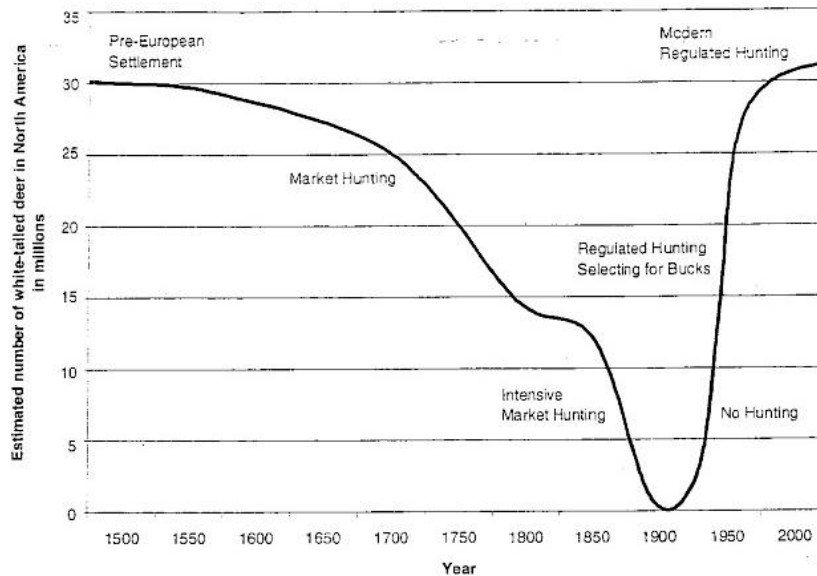


Figure 1: Estimated number of white-tailed deer in North America throughout European settlement (VerCauteren 2003).

Overhunting also decimated the white-tailed deer population in Pennsylvania during the 19th century. Declining much earlier than populations in Kentucky or Mississippi, white-tailed deer in Pennsylvania were reduced to <500 individuals statewide by 1895. Restocking efforts by the Pennsylvania Game Commission began in 1906, nearly thirty years earlier than restocking efforts in Mississippi and forty years earlier than restocking efforts in Kentucky (Kosack 1995). Deer were restocked into Pennsylvania from populations in Kentucky, Maine, Michigan, New Hampshire, New Jersey, North Carolina, and Ohio until 1925 (Fleegle 2016). The Pennsylvania deer population has since rebounded to 1.5 million individuals statewide as of 2009, representing another conservation success for deer in the United States (Pennsylvania Bureau of Forestry 2013). However, no study of the genetic diversity in Pennsylvania white-tailed deer has been conducted. This population continues to flourish 110 years after it rebounded from extreme bottleneck by means of one of the first

documented white-tailed deer translocations in North America. Therefore, a survey of its genetic diversity would provide useful information about the long-term consequences of translocation and conservation strategies for other organisms experiencing current bottlenecks.

This study assessed genetic diversity in the current Pennsylvania deer population 110 years after initial translocation was conducted. Two microsatellite markers originally studied in Anderson et al. (2002), *BM4208* and *Cervid1*, were surveyed in deer samples from across the state. *Cervid1* was chosen because it has been surveyed in most major studies of white-tailed deer, allowing for direct comparison with other populations (DeYoung et al. 2003; Doerner et al. 2005; Kekkonen et al. 2012). *BM4208* was chosen because previous studies show this locus to be independent of *Cervid1*, increasing the robustness of our statistical analyses by ensuring that genotypes at one locus do not influence genotypes at the second locus by recombination (Anderson et al. 2002). Fragment analysis of *BM4208* and *Cervid1* was used to determine the number of alleles, observed heterozygosity (percentage of individuals who are heterozygotes at a locus), and expected heterozygosity to determine the overall genetic diversity within the Pennsylvania white-tailed deer population. These values were compared to other recovered white-tailed deer populations to assess relative success of Pennsylvania translocations. The data were also tested for evidence of a past genetic bottleneck in the population.

Materials and Methods

Sample Collection

A total of 23 ≥ 1 gram white-tailed deer tissue (meat or skin) samples from the 2013-2014, 2014-2015, and 2015-2016 hunting seasons were collected from local hunters in Cameron County (n=1), Clearfield County (n=2), Erie County (n=3), Mercer County (n=2), Potter County (n=1), Warren County (n=3) and from local hunters and a meat processing facility in York County (n=11) (Figure 2). Hunters were contacted through colleague connections and social media broadcasts (i.e., “Facebook status”).

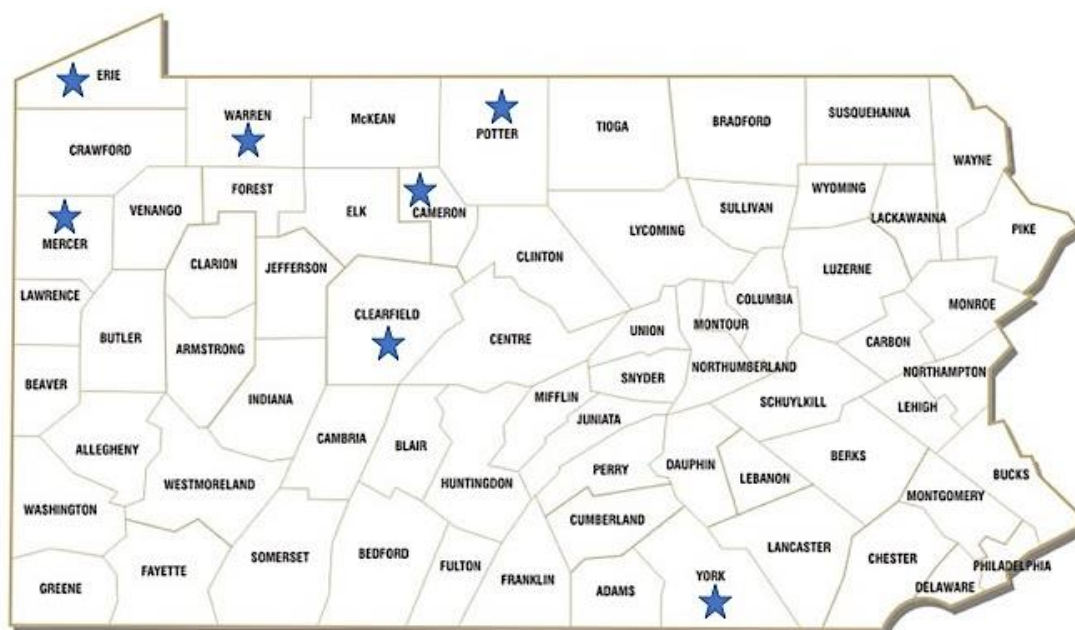


Figure 2: Map of Pennsylvania with sampled counties starred

All hunters were given the same instructions. Deer samples were accepted from past hunting seasons only if they had been continuously frozen at $\leq 0^{\circ}\text{C}$ since butchering. Hunters were also asked to freeze any deer samples killed during the current season at $\leq 0^{\circ}\text{C}$. Hunters cut a small piece of meat/skin and placed it in a plastic bag with information about the sample’s sex, location killed, and date killed. No hunters were financially rewarded for providing samples for this study.

All samples from York County were retrieved by the researcher personally by visiting homes and Godfrey Brothers Meats meat processing facility (York, PA). Samples from all other locations were personally delivered to the researcher on ice in a cooler and then transferred to a permanent freezer to be stored at -20°C until DNA extraction. If hunters offered multiple samples, they had to ensure that each tissue sample never came in contact with another. The researcher ensured that each specimen was received in a separate plastic bag upon delivery. Ground meat samples were rejected since they could contain more than one individual. Samples that violated any of these specifications were not accepted to control for cross-contamination.

DNA Extraction

DNA was extracted using the DNeasy Blood and Tissue Kit, Purification of Total DNA from Animal Blood or Cells Protocol (Spin-Column Protocol) (Qiagen, Valencia, CA). Given the robust nature of muscle connective tissue, each sample was divided into ≥ 10 pieces with a sterile razor blade to maximize surface area for digestion. Since muscle tissue is often transcriptionally active, 20 μL of RNase A (25 mg/ml) was added per sample after lysis. All DNA extractions took place with a negative control to provide a check for contamination. DNA extractions were assessed using Nanodrop Spectrophotometry. Successful DNA extraction was defined as a DNA concentration greater than 10 ng/ μL and a 260/280 ratio of approximately 1.8, as 1.8 indicates pure DNA. Given the limited nature of samples, samples which did not meet these requirements were still tested for amplification. DNA extracts were stored at 4°C until amplification. To aid future sample collection, an unpaired t-test was run in JMP (JMP®, Version 12.0.1 SAS Institute Inc., Cary, NC 2007) to test for any difference in extraction efficiency between meat and skin samples, with a null

hypothesis of no difference between collection methods. For unpaired t-tests, t-values closer to zero signify less deviation from the null hypothesis, and α was set at 0.05 to determine the statistical significance of these deviations.

Amplification of *Cervid1* and *BM4208* loci

The 6-FAM labeled M13 (-21) primer was ordered in a 50 nmol concentration dry and salt-free from Eurofins MWG Operon (Huntsville, AL) for the fluorescent labeling approach laid out by Schuelke (2000). The forward and reverse primers previously published for *Cervid1* and *BM4208* by Anderson et al. (2002) were ordered dry and salt-free from Eurofins MWG Operon (Huntsville, AL) with the specified M13(-21) “tail” attached to the forward primers (Table 1).

Table 1: Primers to be used for the amplification of *BM4208* and *Cervid1* loci (Anderson et al. 2002; Schuelke 2000).

Primer	Primer Sequence ^A	Product (bp)
<i>Cervid1</i>	F: 5' - <u>TGTA</u> AAACGACGGCCAGTAAATGACAACCCGCTCCAGTATC - 3' R: 5' - TCCGTGCATCTCAACATGAGTTAG - 3'	159-193
<i>BM4208</i>	F: 5' - <u>TGTA</u> AAACGACGGCCAGTTCAGTACACTGGCCACCATG - 3' R: 5' - CACTGCATGCTTTTCCAAAC - 3'	142-173
<i>M13(-21)</i>	6-FAM - 5' - <u>TGTA</u> AAACGACGGCCAGT - 3'	N/A

^AUnderlined portion of sequences denote M13(-21) tail specified by Schuelke (2000) for 6-FAM attachment to PCR products.

The 6-FAM labeled M13 (-21) primer was re-suspended in 1x TE buffer pH 7.6 to a concentration of 100 μ M, aliquoted in 50 μ L increments into separate foil-wrapped 1.5mL tubes, and dried using the Savant SPD1010 SpeedVac Concentrator at a vacuum pressure of 5.1 without heat. Dried aliquots were stored in foil-wrapped tubes inside a dark box at -20°C. Forward and reverse primers for the *Cervid1* locus were re-suspended in DNase/RNase-free water to a stock concentration of 100 μ M and then diluted to working concentrations of 1 μ M

and 5 μM , respectively. Forward and reverse primers for *BM4208* were also re-suspended to a stock concentration of 100 μM and then diluted to working concentrations of 2 μM and 5 μM , respectively. Stock primers were stored at -20°C . Diluted primers were stored at 4°C .

The Polymerase Chain Reaction (PCR) consisted of 6.25 μL 2x Type-it Multiplex PCR MasterMix from Qiagen Type-it Microsatellite PCR Kit (QIAGEN Sciences Inc., Germantown, Maryland, United States), 0.6 μL forward primer (0.6 μM for *Cervid1*, 1.2 μM for *BM4208*), 0.95 μL reverse primer (4.75 μM for both loci), 0.95 μL 6-FAM labeled M13 (-21) primer (4.75 μM), 1-2 μL DNA template (~ 1.12 ng/ μL for *Cervid1*, ~ 2 ng/ μL for *BM4208*), and 1.75-2.75 μL water for a total reaction volume of 12.5 μL . All reactions were run alongside a negative control to check for contamination.

Thermocycler conditions for PCR amplification of *Cervid1* and *BM4208* loci were as follows: initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation (95°C for 30 seconds), annealing ($60^{\circ}\text{C}/59^{\circ}\text{C}$ [*Cervid1/BM4208*] for 90 seconds), and elongation (72°C for 30 seconds); followed by 8 cycles of denaturation (94°C for 30 seconds), annealing (53°C for 45 seconds), and elongation (72°C for 45 seconds); with a final extension at 60°C for 30 minutes. These conditions were specified by the Qiagen Type-It Microsatellite PCR Kit (QIAGEN Sciences Inc., Germantown, Maryland, United States). For *Cervid1*, the annealing temperature for the first 30 cycles was specific to thermocycler conditions used in Shaw et al. (2006). For *BM4208*, the annealing temperature for the first 30 cycles was experimentally determined. PCR products were held at 4°C on the thermocycler until they were moved to a 4°C refrigerator for storage in a foil-wrapped tray.

PCR success was assessed by checking the products and negative control via gel electrophoresis on a 1% agarose gel with 6x orange loading dye and a low mass DNA ladder

(Invitrogen Life Technologies, Grand Island, NY), running at 70 V for 45 minutes. PCR success was defined as a bright band in the expected size range (Table 1). Contamination was denoted by the presence of an amplicon in the negative control. Amplicons present at >2000 bp in the negative control were a result of the 2x Type-it Multiplex PCR MasterMix becoming contaminated during optimization. This contamination was noted, but did not disqualify samples from fragment analysis. Successful reactions were diluted 1:10 in molecular grade water, placed in a foil-wrapped falcon tube, and shipped via FedEx Express-2Day Shipping to Yale University DNA Analysis Facility (New Haven, CT) for fragment analysis using the New Gel Co Liz-500 size standard.

Genotype Determination

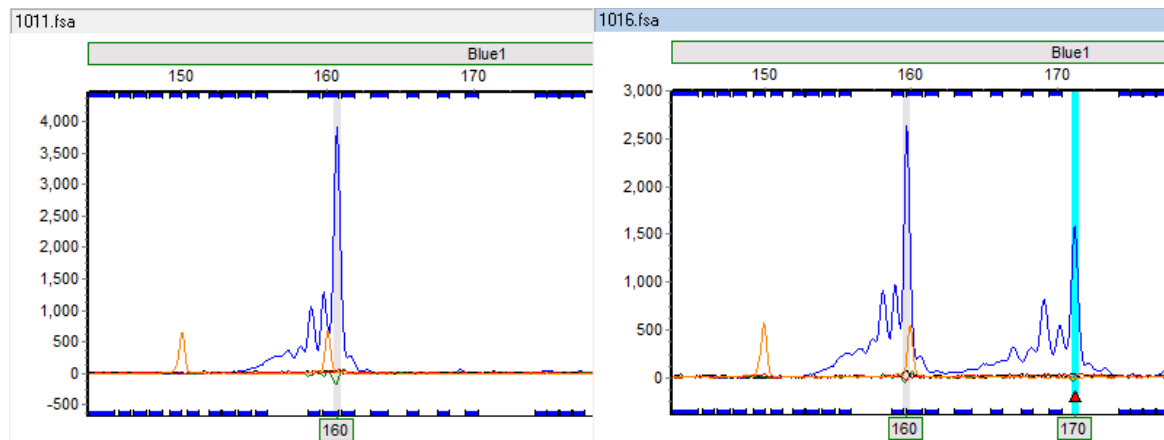


Figure 3: Peak data displayed on GeneMarker V2.6.7 for two samples at *BM4208*. Sample 1011 (left) is homozygous (142bp,142bp) while sample 1016 (right) is heterozygous (142bp,152bp).

The raw fragment analysis data obtained from Yale was analyzed using GeneMarker V2.6.7 (Demo) developed in State College, PA (Holland and Parson 2011). FSA files were imported into GeneMarker and analyzed by the “Run” command, specifying “Fragment (Animal)” for analysis type and using the AFLPPanel Panel and GS-500 size standard. The true allele length was called by the tallest, longest peak, minus eighteen base pairs to account for the M13(-21) primer. The presence of a single large peak within the expected region

denoted homozygosity while two large peaks denoted heterozygosity (Figure 3). Samples with >2 peaks were considered unsuccessful. Since each allele has a characteristic peak profile, samples with the same allele were viewed “in alignment” to check that peak profiles matched. This strategy provided a check on incorrect genotype calls (Figure 4).

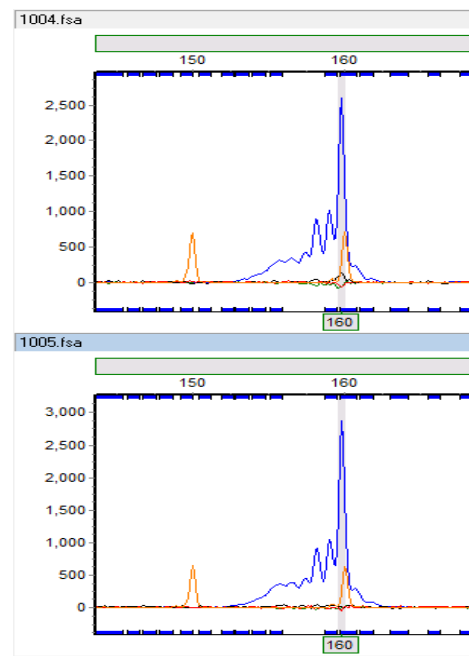


Figure 4: Peak data “in alignment” for samples 1004 (above) and 1005 (below) to check that peak profiles match for allele 142bp at *BM4208*.

Genetic Diversity

Genotypes for both loci were coded in GENEPOP input file format and imported into GENEPOP 3.4 (Raymond and Rousset 1995) to test linkage disequilibrium between the two loci. Given that the two loci were found to be unlinked, genotype data were imported into GENODIVE 2.0 (Meirmans and Van Tienderen 2004) to calculate allele number, allelic frequencies, deviation from Hardy-Weinberg equilibrium (HWE), and overall observed heterozygosity of the population. The population was tested for heterozygote excess/deficiency by one-tailed U-tests in GENEPOP 3.4. GENEPOP 3.4 was also used to calculate expected and observed heterozygosity at each locus. Genotype data were converted

to FSTAT format in GENODIVE 2.0 and imported into FSTAT 2.9 (Goudet 1995) to determine overall allelic richness and allelic richness at each locus.

Genetic Structure

Samples from York County, PA (n=11) and northwestern PA (n=12) were sorted into separate groups. GENODIVE 2.0 was used to calculate an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) between York County, PA and northwestern PA to detect the presence of genetic structure within the state population.

Evidence of Genetic Bottleneck

Genotype data were imported into BOTTLENECK 1.2 (Cornuet and Luikart 1996) to examine our data for a history of population decline. This program detects population bottleneck by the following assumption: in a population at mutation-drift equilibrium, there is an equal probability of heterozygosity excess or deficit at each locus insofar as effective population size remains constant. Therefore, a deviation from this probability indicates recent population size decline (i.e., bottleneck). The Two-Phase mutation model (TPM) was assumed, as this model is recommended for microsatellite loci (Cornuet and Luikart 1996). The parameters for TPM were set at 30% variance and 70% probability, following protocol for white-tailed deer microsatellite analysis laid out in Kekkonen et al. (2012). Given that our dataset only contained 2 loci, the Sign test and Wilcoxon test were chosen for statistical analysis, omitting the Standardized Differences test which requires a minimum of 20 loci. The Sign test compares paired differences between observed heterozygosity and expected heterozygosity by individual over 1000 replications of possible combinations of individuals in mutation-drift equilibrium. The Wilcoxon test is a non-parametric version of the Sign test, accounting for non-normal allele distribution. For both tests, α was set at 0.05 to determine

the statistical significance of deviations from mutation-drift equilibrium. BOTTLENECK 1.2 was also used to test for normal L-shaped distribution of allele frequencies. Allelic frequencies were plotted on a frequency distribution histogram for qualitative evaluation. Kekkonen et al. (2012) provides allele frequency data of *Cervid1* for a severely bottlenecked population in Finland, so this data was also plotted on a frequency distribution histogram for comparison.

Results

DNA Extraction

DNA was successfully extracted from all 23 samples of white-tailed deer tissue. DNA concentrations ranged from 5.5-93.5 ng/ μ L. Purity values ranged from 1.43-2.79 (Appendix A). No difference in DNA concentration ($t(21) = -0.871$, $p=0.394$) or purity ($t(21) = 1.45$, $p=0.170$) was observed between DNA extractions of skin and muscle samples.

Table 2: Genotypes of *Cervid1* and *BM4208* in twenty-three deer samples

ID	Location	Cervid1	BM4208 ^A
1001	York	170,172	-
1002	Potter	172,172	-
1003	York	177,187	-
1004	York	177,187	142,160
1005	York	187,189	142,166
1006	Mercer	159,170	170,176
1007	Mercer	170,170	162,176
1008	Cameron	157,181	-
1009	York	187,189	152,152
1010	York	171,197	152,166
1011	York	173,189	142,142
1012	Warren	170,179	166,166
1013	Warren	181,181	158,158
1014	Warren	171,177	-
1016	York	185,187	142,152
1017	York	185,185	152,160
1018	York	181,181	142,142
1019	York	171,197	166,166
1027	Clearfield	179,185	170,176
1028	Clearfield	170,170	168,170
1029	Erie	171,181	146,178
1030	Erie	173,173	168,178
1031	Erie	177,181	-

^A “-” denotes missing data

Genetic diversity

Amplification was successful in 23 samples at the *Cervid1* locus and 17 samples at the *BM4208* locus. Seven individuals were found to be homozygous at *Cervid1*, six individuals were found to be homozygous at *BM4208*, and 2 individuals were found to be homozygous at both loci (Table 2). For both loci, observed heterozygosity was lower than expected heterozygosity, and neither locus was found to be in Hardy-Weinberg Equilibrium (HWE). This result was supported by a U-test ($p < 0.001$ for both loci), which detected significant heterozygote deficiency in the population (Table 3). A total of twenty-five alleles were detected in the population, with a mean allelic richness of 12.149 (rounded to 12) and an overall heterozygosity of 0.672. No linkage disequilibrium was detected between the two loci ($p = 1.00$), so we rejected the alternative hypothesis that genotypes at *Cervid1* are dependent (e.g. “linked”) on genotypes at *BM4208*. All subsequent tests assumed independent loci, so this determination increased the robustness of our statistics.

Table 3: Population-level statistics of genetic variability in Pennsylvania white-tailed deer at 2 microsatellite loci

Locus ^A	Na ^B	Range (bp) ^C	H _o ^D	H _e ^E	HWE ^F	Ar ^G
<i>Cervid1</i>	13	157-197	0.696	0.922	$p = 0.004$	12.298
<i>BM4208</i>	12	142-178	0.647	0.914	$p = 0.001$	12.000

^ALocus name, ^BNumber of alleles, ^CSize range of alleles, ^DObserved heterozygosity (H_o),

^EExpected heterozygosity (H_e), ^FResults of Hardy-Weinberg test (HWE), ^GAllelic richness (A_r)

Genetic Structure

No genetic differentiation was detected ($F_{ST} = 0.041$, $p = 0.029$) between deer in York County, PA and northwestern PA (Table 4). Though this minor F_{ST} -value was statistically significant, F_{ST} -values between 0.00-0.05 denote little to no genetic differentiation between groups (Balloux and Lugon-Moulin 2002).

Table 4: Results AMOVA between deer in York County, PA and northwestern PA

	Df ^A	% Var ^B
Among Population	1	4.1
Within Population	23	95.9
Total	24	--

Fst Value: 0.041

P-value: 0.029

^ADegrees of freedom, ^BPercent variation

Evidence of Genetic Bottleneck

Results from BOTTLENECK 1.2 did not support evidence of recent population decline. The population was found to be in mutation-drift equilibrium at both loci by the Sign Test ($p=0.350$) and Wilcoxon test ($p=0.250$). Furthermore, there was no evidence of a mode shift in the allele frequency distribution. The graph featured a strong mode at the lowest allele frequency class, characteristic of a population which has not undergone a genetic bottleneck (Figure 5).

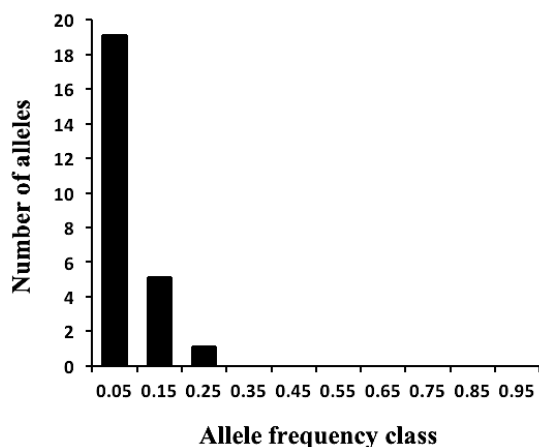


Figure 5: Allelic frequency distribution of twenty-five alleles from two polymorphic loci found in twenty-three Pennsylvania deer samples.

Discussion

White-tailed deer in Pennsylvania experienced a severe population bottleneck at the turn of the 19th century as a result of overhunting, reducing the statewide population to <500 individuals by 1895 (Kosack 1995). Following initial restocking efforts in 1906, white-tailed deer quickly rebounded past historic, pre-European settlement levels and soon required managed hunting to keep the population in check (Kosack 1995; VerCauteren 2003; Fleegle 2016). Despite this history of population reduction and resurgence, our results suggest that the Pennsylvania white-tailed deer herd exhibits a healthy degree of microsatellite genetic diversity, qualitatively comparable to other recovered deer populations (Table 5). Furthermore, our results indicate that either the drastic population reduction over 110 years ago was not accompanied by a genetic bottleneck at these loci or that the translocation efforts effectively recovered the genetic diversity of any genetic bottleneck that existed at the time.

Table 5: Number of alleles detected (N) and observed heterozygosity (H_o) in surveyed deer populations at markers *Cervid1* (A) and *BM4208* (B).

A.			B.		
<i>Cervid1</i>			<i>BM4208</i>		
Population	N	H_o	Population	N	H_o
Pennsylvania (n=23)	13	0.696	Pennsylvania (n=23)	12	0.647
Oklahoma (n=72) ^A	14	0.833	Oklahoma (n=72) ^A	13	0.946
Kentucky (n=332) ^B	24	0.733	Kentucky (n=332) ^B	26	0.645
Finland (n=72) ^C	6	0.719			
Canada (n=2088) ^D	27	0.840			

^AAnderson et al. (2002) ^BDoerner et al. (2005) ^CKekkonen et al. (2012) ^DCullingham et al. (2011)

Genetic Diversity

Since *Cervid1* and *BM4208* consistently rank among the most polymorphic loci analyzed in deer (Anderson et al. 2002; Doerner et al. 2005; Xochitl et al. 2012), we compared populations solely based on locus-specific heterozygosity values, rather than on the average heterozygosity for all loci sampled in a population. This approach mitigated the

possibility of overestimating the comparative genetic diversity of the Pennsylvania population to populations where less polymorphic loci were included in the panel. Qualitatively, the observed heterozygosity of *Cervid1* and *BM4208* in Pennsylvania deer (0.672) is similar to the value found in Kentucky (0.689) but lower than the value found in Oklahoma (0.890) (Figure 6). This similarity is interesting considering that Kentucky deer were part of the initial translocations into Pennsylvania in 1906 (Kosack 1995).

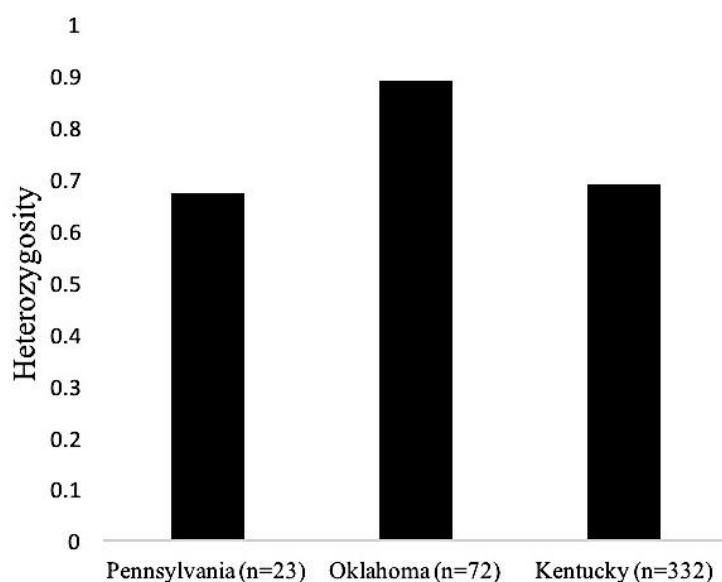


Figure 6: Average observed heterozygosity of *Cervid1* and *BM4208* in Pennsylvania, Oklahoma (Anderson et al. 2002), and Kentucky (Doerner et al. 2005) deer populations

Despite Oklahoma having a higher observed heterozygosity at *Cervid1*, the overall heterozygosity in Kentucky (0.657) and Oklahoma (0.608) are similar (Anderson et al. 2002; Doerner et al. 2005). The Pennsylvania survey needs to be expanded to include roughly 15 loci but we expect its overall heterozygosity to be comparable with these two populations, which have an extensive degree of microsatellite genetic variation (Doerner et al. 2005; Brommer et al. 2015). A good indication of this prediction is revealed through a comparison of allelic richness (allele number controlling for sample size) of *Cervid1* in Oklahoma (14) and Pennsylvania (12) (Table 3). Oklahoma has greater heterozygosity at this locus, but the

populations are comparable in terms of allelic richness. Allele number of *BM4208* is also very similar in Pennsylvania and Oklahoma (Table 5).

Deer in Finland provide an excellent comparison for genetic diversity. This population was founded from just 4 deer (3 females and 1 male) in 1934 and now contains 40,000-50,000 deer (Kekkonen et al. 2012). The isolation of this population is well-documented, and provides an extreme example of bottleneck, since only one male founded the entire current population. By observed heterozygosity, it is comparable to Pennsylvania and other previously published studies (Table 5). Yet, with a sample size identical to the survey in Oklahoma, a study of this Finland deer population found an allelic richness of only six at *Cervid1*, while this value was found to be twelve and fourteen in Pennsylvania and Oklahoma, respectively (Anderson et al. 2002; Kekkonen et al. 2012). In a more recent study of 422 Finnish deer from the same herd, the allelic richness of *Cervid1* was found to be seven (Brommer et al. 2015), illustrating that nearly the same number of alleles were detected even after the sample size was expanded by 586%. Twice as many alleles were found in the Pennsylvania sample compared to the Finnish sample, even though Brommer et al. (2015) sampled 18x as many deer as the current study in Pennsylvania. This difference is striking, demonstrating the drastic effects of bottlenecks on allelic diversity and supporting our claim that Pennsylvania genetic diversity has been recovered. Furthermore, this comparison sheds light on the limitations of using solely observed heterozygosity to assess genetic diversity in a population, strengthening the power of our comparison of allelic richness between Pennsylvania and Oklahoma.

Compared to a large study of deer across Alberta, British Columbia, and Saskatchewan, observed heterozygosity of *Cervid1* is higher in Canada than Pennsylvania

(Table 5). The overall heterozygosity in Canada (0.677) was also slightly greater than Oklahoma or Kentucky. Though Canadian deer experienced a similar population reduction around 1900, the nature of their resurgence was slow and expansive, with geographic range expanding as the population grew (Cullingham et al. 2011). The Canadian deer population mainly recovered in uncut forests with harsh winters, which drastically reduced survival compared to deer in the United States. They also shared this habitat with moose and mule deer, which are adapted to colder climates (Wishart 1984). Cullingham et al. (2011) suggest that this slow-moving population expansion maintained heterozygosity. In contrast, the Pennsylvania population expanded exponentially in size and density after 1906, taking advantage of both the plentiful food found in the decimated forests (i.e., twigs, brush) and the lack of predation (Fleegle 2016). Therefore, the difference in heterozygosity between these populations may correspond to distinct resurgence histories, suggesting that not all bottleneck events are the same. Preservation of genetic diversity may depend on the speed and density at which the population recovers.

Lastly, the observed heterozygosity of the Kentucky population at markers *RT9* and *BM1225* was equivalent to deer in northern New England, a population untouched by the overhunting which occurred in Mid-Atlantic and Southern states (Doerner et al. 2005). These markers need to be analyzed in the Pennsylvania samples to make a direct comparison. However, we can infer that Pennsylvania deer display a similar level of diversity to deer in the un-exploited New England region because of the similarity in observed heterozygosity between Kentucky and Pennsylvania populations. This inference supports our argument that genetic diversity has been preserved. It's also worth noting that deer from the New England area (e.g., Maine) were part of deer translocations into Pennsylvania in 1906 (Fleegle 2016).

Moreover, Doerner et al. (2005) set a precedent in the literature for comparing populations based on only two loci, lending power to the comparisons we have made between previous studies and the current study using just *Cervid1* and *BM4208*.

Hardy-Weinberg Equilibrium

Our finding that both loci are out of the Hardy-Weinberg equilibrium (HWE) is not uncommon for deer populations in North America (Table 3). Heterozygote deficiency is documented as the cause of these deviations in all cases, corresponding to our results (Doerner et al. 2005; DeYoung 2003; Xochitl et al. 2012; Anderson et al. 2002). These findings can be explained by deer's typical polygynous mating behavior where a few dominant males mate with the same group of females each year, violating the HWE expectation of random mating (Hirth 1977). However, recent molecular work has suggested a breakdown in this traditional view, wherein younger males gain access to females and multiple-paternity litters occur frequently (DeYoung et al. 2002). Turner et al. (2016) suggest that deer populations take part in this atypical behavior more frequently when polygynous breeding requires too much effort energetically with respect to the environment.

This finding in Turner et al. (2016) may explain why the deer population in Finland is found to be in HWE, a phenomenon not found in North American deer (Kekkonen et al. 2012; Brommer et al 2015). Despite an exponential growth pattern similar to Pennsylvania deer, Finnish white-tailed deer are not a native species to Finland (Kekkonen et al. 2012). Winters are so harsh that Finnish hunters have established ~76,000 feeding stations across the country to help deer and other imported game species survive the winter. Reindeer, moose, and three other native species of deer all compete for the same food and land use. Furthermore, natural predators like bears and wolves thrive in this environment (Hunters'

Central Organization 2009). In these conditions, dominant male deer may find it hard to defend a harem of females, allowing many younger males access to females. This mating pattern more closely resembles random mating, which might explain why most loci are found to be in HWE in Finland but not in North American deer populations where conditions support polygyny. Our hypothesis could be further tested by assessing Hardy-Weinberg equilibrium in Canadian deer, which also experience harsh winters and competition for resources with other Cervids (Wishart 1984).

Genetic Structure

Our results suggest that deer translocations at the beginning of the 20th century did not result in genetic differentiation across the state, illustrated by the little variation found between deer in York County, PA and northwestern PA (Table 4). These results concur with studies in Canada, Kentucky, and Mississippi, where weak genetic differentiation was found across comparably sized areas (Cullingham et al. 2011; Doerner et al. 2005; DeYoung et al. 2003). Furthermore, DeYoung et al. (2003) concluded that the translocations themselves may be responsible for the lack of genetic differentiation across statewide populations, attributing them to a long-term influence on the genetic structure of some Mississippi deer populations. Studies in the northeast and southwest regions of Pennsylvania are needed to confirm these findings (Figure 2).

In studies of white-tailed deer, geographic barriers are usually the main contributors to genetic differentiation between groups. Most of the variation between subpopulations in the study of deer across Canada was attributed to the Rocky Mountains. The same study found that geographic distance between groups was the strongest predictor of genetic differentiation (Cullingham et al. 2011). A study of Mexican deer unexpectedly found that

grouping deer by geographic region produced higher F_{ST} -values than grouping deer by their genetically-distinct subspecies. Still, F_{ST} -values between groups with the starkest differences were never higher than 0.133, suggesting that deer populations are generally genetically homogenous even across large, seemingly-disconnected regions (Xochitl et al. 2012). In conjunction with these findings, our results strongly reject that the Appalachian Mountains pose a geographic barrier to gene flow in the Pennsylvania deer population, although a wider sample collection and loci panel are needed to support this theory more fully (Figure 7).

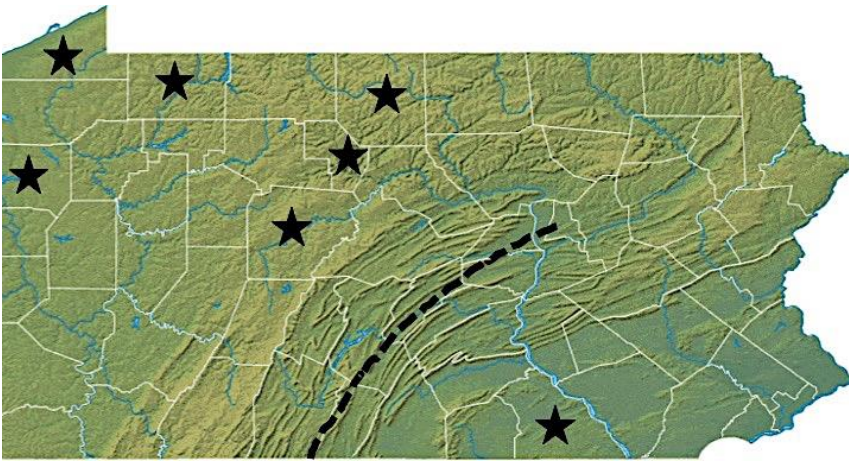


Figure 7: The Appalachian Mountains (dotted line) are a potential geographic barrier between sample locations (stars) in northwestern PA and York County, PA.

Evidence of Genetic Bottleneck

In concurrence with other previously studied populations of white-tailed deer, our results demonstrate that the population bottleneck of Pennsylvania deer over one hundred years ago was not accompanied by a genetic bottleneck. Though preliminary because of small sample size and a limited number of loci analyzed, this claim is based on three pieces of evidence. First, the results of the sign test and Wilcoxon test showed no evidence of a recent population decline via heterozygosity excess. These tests are accepted as strong indicators of genetic bottlenecks across a variety of taxa (Kuo and Janzen 2004; Kekkonen et al. 2012; Brown et al. 2007). Although the M-ratio test is considered the gold-standard of

bottleneck testing in population genetics because of its sensitivity up to 100 overlapping generations, this test requires a minimum of 5 loci to give meaningful results and at least 15 loci to have statistical power (Garza and Williamson 2001). For white-tailed deer specifically, Brommer et al. (2015) found that >10 microsatellites are needed to conduct a survey with statistical power. Therefore, future studies should expand our survey to more loci to test whether the M-ratio test concurs with the results from BOTTLENECK 1.2.

Second, observed heterozygosity and allelic richness of *Cervid1* and *BM4208* are similar to these values in populations which show no evidence of genetic bottleneck (DeYoung et al. 2003; Doerner et al. 2005; Cullingham et al. 2011). Pennsylvania, Mississippi, Kentucky, and Canada deer populations share an analogous history of overhunting, population bottleneck, and resurgence, though Canada differs from United States' populations in its manner of recovery. Moreover, the Kentucky population has comparable genetic diversity to groups of deer with histories of minimal overhunting, representing pre-bottleneck levels of diversity (Doerner et al. 2005).

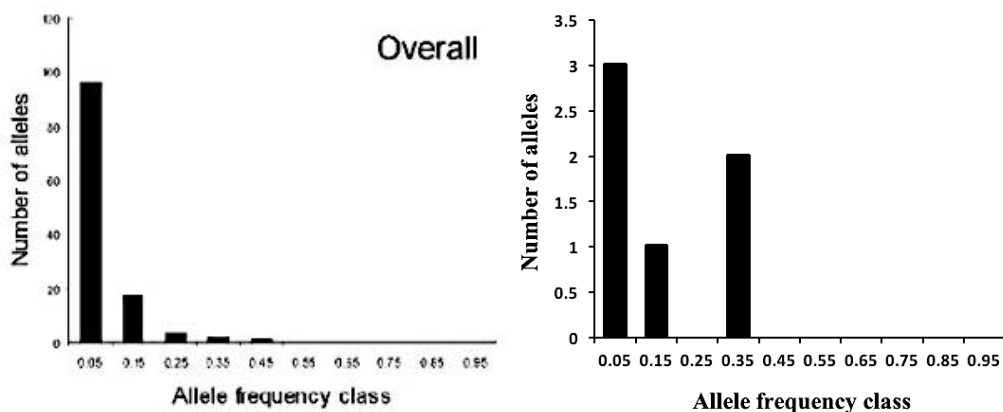


Figure 8: Allele frequency distribution of deer in Kentucky (left) compared to allele frequency distribution of deer in Finland (Doerner et al 2005; Kekkonen et al 2012).

Third, no mode shift was detected in the frequency distribution histogram, consistent with a population under mutation-drift equilibrium (Doerner et al. 2005). Additionally, a

comparison between the allele frequency distribution of Kentucky deer and Finnish deer provides a striking contrast between a population with no evidence of genetic bottleneck (Kentucky) and a population where the founder effect/bottleneck event is known (Finland) (Figure 8). Upon visual inspection, the frequency distribution of Pennsylvania deer more closely resembles the frequency distribution of Kentucky deer than the bottlenecked Finnish population (Figure 5).

Management Implications

Our findings provide evidence that the translocation of deer into Pennsylvania was a successful conservation effort in both increasing population size and maintaining genetic diversity. Though the genetic diversity of deer populations has already been surveyed in other states, this study is unique because it documents genetic diversity one hundred and ten years after initial translocation, providing the longest-term genetic evaluation of translocation which exists in white-tailed deer literature. More generally, our study suggests that translocation is a sustainable means to preserve genetic diversity in threatened populations while also growing population size.

In consensus with other studies, we find no long-term side effects of translocation on the genetic structure of white-tailed deer populations. However, more sampling and a broader panel of loci are needed to establish this finding conclusively. Despite geographic barriers often causing genetic structure in deer populations, our results suggest that the Pennsylvania Game Commission should not view the Appalachian Mountains as a geographic barrier to gene flow in the deer population, though, studies should be carried out to see if they have created genetic structure in other recovering statewide species, such as the American Black Bear (Ternent and Bureau of Wildlife Management 2001). With the emergence of diseases

like Chronic Wasting Disease, the Pennsylvania Game Commission should note that a lack of genetic differentiation may signal intermixing of local herds, possibly increasing transmissibility of wildlife diseases that could harm both deer and humans (Cullingham et al. 2011).

Researchers should be aware that microsatellites are simply markers of genetic diversity at non-coding regions in the genome and do not always reveal a similar trend in phenotypic traits needed for survival in the wild. Heterozygosity has been positively correlated with increased body mass in deer, but not with other indicators of fitness like jaw size or antler score (Brommer et al. 2015). These findings indicate that jaw size and antler score are possibly influenced by environmental conditions, rather than being a strict result of genetics. We propose a survey of genes where genetic diversity is directly related to phenotypic fitness, such as the Major Histocompatibility Complex (Sommer 2005). If studies of this gene within the Pennsylvania deer population exhibit the same level of genetic diversity as microsatellite regions, we can imply that phenotypic fitness was also preserved by translocation.

Though our findings suggest the long-term success of translocation in preserving genetic diversity, it should be noted that the nearly exponential growth of the Pennsylvania deer population also likely contributed to this preservation. At the turn of the 20th century, Pennsylvania's landscape was exceptionally well-suited to deer, with a virtually limitless food supply and no natural predation. Along with translocations, this habitat is largely credited with deer resurgence across the state (Fleegle 2016). As an r-selected species reproducing in overlapping generations, deer have been shown to expand genetic diversity after severe founder effect simply as a product of their massive reproductive potential,

possibly because overlapping generations decrease the risk of genetic drift (DeYoung et al. 2003; Kekkonen et al. 2012; Brommer et al. 2015). Yet, this reproductive potential is likely only realized when conservation efforts are aimed at maximizing food availability, decreasing predation, and expanding suitable habitat (Hunters' Central Organization 2009).

Translocation is not likely to be sufficient for genetic rescue if not complemented with a management program aimed at habitat restoration and protection. Furthermore, many threatened animals in need of translocation are k-selected, having small litters and taking longer to reach sexual maturity, like the American Black Bear (Ternent and Bureau of Wildlife Management 2001). In these species, overlapping generations are less likely to occur and genetic drift will contribute heavily to population genetics (Hartl and Clark 2006). However, when ecosystem conditions are suitable, translocations prove to be an invaluable tool in preserving the genetic diversity of threatened populations. The historic translocations discussed in this paper likely contribute to the genetically healthy deer population found across the United States today. For any species currently at risk for local extinction, translocation should be highly considered as a long-term conservation strategy to save these populations.

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Appendix

Appendix A: DNA extraction results of twenty-three white-tailed deer tissue samples.

ID	Type	Location	DNA Concentration (ng/uL)	Purity (260/280)
1001	Skin	York	19.7	1.62
1002	Skin	Potter	27.0	1.76
1003	Skin	York	86.5	1.50
1004	Muscle	York	56.2	1.90
1005	Muscle	York	93.5	1.64
1006	Muscle	Mercer	35.6	2.00
1007	Muscle	Mercer	46.5	1.59
1008	Muscle	Cameron	18.1	1.71
1009	Muscle	York	25.4	1.88
1010	Muscle	York	22.6	1.63
1011	Muscle	York	37.8	1.84
1012	Muscle	Warren	46.5	1.94
1013	Muscle	Warren	28.8	1.80
1014	Muscle	Warren	25.8	1.72
1016	Muscle	York	13.5	2.79
1017	Muscle	York	8.80	1.43
1018	Muscle	York	11.8	2.43
1019	Muscle	York	5.50	2.64
1027	Muscle	Clearfield	49.7	1.89
1028	Muscle	Clearfield	42.1	1.87
1029	Muscle	Erie	23.0	1.72
1030	Muscle	Erie	32.5	1.93
1031	Muscle	Erie	23.7	2.00