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Genetic Diversity of White-Tailed Deer Populations in Southwestern Pennsylvania and the Development of a Forensics Panel

Melanie Quain

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GENETIC DIVERSITY OF WHITE-TAILED DEER POPULATIONS IN SOUTHWESTERN
PENNSYLVANIA AND THE DEVELOPMENT OF A FORENSICS PANEL

A Thesis

Submitted to the Bayer School of Natural and Environmental Sciences

Duquesne University

In partial fulfillment of the requirements for
the degree of Master of Science

By

Melanie R Quain

August 2019

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Melanie Quain

2019

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PENNSYLVANIA AND THE DEVELOPMENT OF A FORENSICS PANEL

By

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ABSTRACT

GENETIC DIVERSITY OF WHITE-TAILED DEER POPULATIONS IN SOUTHWESTERN PENNSYLVANIA AND THE DEVELOPMENT OF A FORENSICS PANEL

By

Melanie R Quain

August 2019

Thesis supervised by Dr. Jan E. Janecka

During the 20th century, white-tailed deer (*Odocoileus virginianus*) became scarce, prompting conservation efforts by hunters and wildlife managers with the goal to recover this species. Various strategies were implemented including reintroductions from areas that still had large deer populations, developing bag limits, seasonal restrictions, and habitat management. These efforts were highly successful across the United States.

Today, white-tailed deer are one of the most abundant and widely-distributed large-bodied mammals in North America. However, there are several important management concerns. In numerous states, including Pennsylvania, CWD negatively impacts deer populations and has become a major health concern. When studying factors of disease spread, population genetics has been proven useful when observing patterns of gene flow to determine the movement of infectious individuals. In addition, poaching of deer is a recurrent problem in many states and reduces the ability to effectively manage this species.

Illegal harvest of wildlife can directly impact a populations abundance, distribution, sex ratios, remove trophy deer, and alter age structure. The severity of wildlife crime is difficult to accurately assess as many offenses go undetected. Poaching often occurs in remote and isolated areas that have limited monitoring. The advancement of forensic science practices is necessary in combating these illegal activities given their high estimated frequency and its inherent threat to species. Forensic science methods applicable to the enforcement of wildlife legislation largely focus on the use of DNA barcoding and fingerprinting to identify species and individuals among samples collected at a crime scene

Microsatellite loci have been proven useful for the identification of individuals, determination of kinship, assignment of migrants to source populations, estimation of gene flow between populations, and examination of geographic variation among a species. The purpose of this thesis was to evaluate the genetic variation within the white-tailed deer populations in southwestern Pennsylvania using seven microsatellite loci and use this information to develop a molecular panel for forensics applications. A total of 82 road-killed and legally harvested white-tailed deer were sampled throughout the region. The allele frequencies, observed heterozygosity, expected heterozygosity, and probability of identity were calculated for each microsatellite loci. All loci were found to be highly variable and effective for studying population parameters in southwestern Pennsylvania deer and estimating dispersal patterns among wildlife management units that will impact the spread of CWD. Seven loci were selected for a forensic microsatellite that yielded an overall probability of identity of less than 1 in a billion. This was successfully applied to match 6 blind control samples and subsequently 2 poaching cases analyzed for the Pennsylvania Game Commission. This panel will likely be effective for population genetic studies and forensic analysis in white-tailed deer throughout the state of Pennsylvania.

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Chapter One

A Literature Review of White-Tailed Deer (*Odocoileus virginianus*) and Wildlife DNA Forensics in the U.S.

1.1. *The Natural History of White-tailed Deer in the State of Pennsylvania*

The white-tailed deer (*Odocoileus virginianus*) is the most abundant large-bodied North American land mammal and has been an important part of human culture for thousands of years. Though deer populations are abundant in present day, beginning in the mid 1800s, the white-tailed deer had suffered a severe population decline and nearly faced extirpation [28]. Deer provided food, clothing, and tools for growing communities [90] and as communities grew, the wildlife surrounding populated areas began to disappear [72].

Agricultural needs began to grow, and clear-cutting forest area for field space severely impacted local wildlife communities [8], furthermore, the lumber industry was on the rise which added to an increase in tree harvesting in Pennsylvanian forests [90]. By 1895, nearly all of the state's abundant game species had disappeared. The concern over the severe drop in wildlife populations birthed the formation of the Pennsylvania board of Game Commissions, now known as the Pennsylvania Game Commission. Though this agency worked hard to conserve and rebuild wildlife populations, the PGC struggled during its first few years [45] due to limited economic resources, not enough individuals to enforce game laws, and little support from local communities [44].

Beginning in the early 20th century, more organized conservation efforts were initiated [26]. Wildlife managers and conservation-minded sportsmen sought out to protect and increase deer populations not only in Pennsylvania, but throughout the entire United States [1]; bag limits were devised and enforced, shorter hunting seasons were put into action, and buck-only seasons began in order to protect the recovering herds [101]. An extensive

restocking program was also implemented, and deer were translocated from various source populations ^{[32] [63] [73]}.

Sportsmen have played a vital role in the protection and growth of deer populations in the United States ^[25]. In addition to wildlife managers implementing new hunting regulations, sportsmen bought land where deer populations could be protected and grow ^[90].

Subsequently, in many areas, white-tailed deer were able to naturally repopulate in the United States. Since their recovery, white-tailed deer abundance rapidly increased in many diverse regions including regenerated forests, farmland, rural townships, and even urban areas. In present day, it is estimated that there are 20 to 25 million white-tailed deer living the United States, with 16 subspecies classified by taxonomists ^[2] (Table 1).

Today, sportsmen continue to be an essential part of wildlife management. Their contributions provide funding for conservation and management programs, and also for the local economy ^[37]. Wildlife management is expensive, and money is needed in order to carry out programs by state agencies including surveying populations, developing and enforcing annual harvest regulations, monitoring the deer populations for CWD, leasing land for public access, and maintaining roads and services in state parks and forests. The majority of this funding comes from sportsmen ^[44]. These funds generated by hunters also benefit other wildlife, in addition to game species, and thus are an essential part of the states operating budget ^[25].

1.2. The Genetic Structure of White-Tailed Deer in the United States

Genetic analysis can be used to estimate many population parameters including gene flow and dispersal. In addition, it can provide information on mating systems, social behavior, phylogeography, fine-scale structure, and the dynamics of a population ^[15].

After being once nearly decimated in the United States, white-tailed deer have since recolonized many areas of the country. In species that have undergone severe demographic bottlenecks, it is expected there will be genetic variation and substantial differentiation among populations ^[52]. However, studies of natural and translocated white-tailed deer in the eastern United States have shown high levels of genetic diversity and low levels of differentiation among regions ^[15]. This is likely due to the translocations that have occurred and the substantial population expansion that limited the effects of genetic drift.

DeYoung et al. (2003) reported high levels of allelic diversity and heterozygosity in Mississippi, where white-tailed deer recovery programs, including translocations, had begun in the early 20th century ^[27]. Data suggested that the populations have retained historical bottlenecks and display significant differentiation that was not consistent with the populations' geographic distribution ^[27]. In addition, a study done on Kentucky white-tailed deer populations, revealed high levels of allelic diversity, heterozygosity, and divergence among regions that also were not consistent with the geography ^[30].

Researchers are also able to use genetic data to determine the risk of disease transmission in a population by observing gene flow and genetic structure ^[90]. From a wildlife management standpoint, factors that influence disease in free-ranging populations include: population density, environmental changes, movement of pathogens, land-use changes, social pressures affecting disease management, feeding and baiting and other artificial management activities that enhance the risk for disease introduction and establishment ^[24]. Beginning in the early 1980s, CWD became a critical disease threat as it appeared in free-ranging white-tailed deer and elk populations ^[55]. CWD is a TSE that involves the transmission of a proteinaceous infectious particle (i.e., a prion) and is the only one known to infect wild populations ^[75]. The prevention of further spread, and the eradication of this disease is an important goal for wildlife managers due to the potential

long-term negative effects it has on white-tailed deer and human health ^[19]. CWD is actively managed in most areas, but the disease is difficult to control due to its extended incubation period, the difficulty in detecting infected individuals, and the lack of vaccines or treatment ^[102].

Estimating gene flow allows for researchers to predict the movement of infected individuals within areas ^[24]. To provide an example, by using a landscape genetics approach, researchers can identify corridors between structured populations that are influenced by ecological and spatial factors ^{[17] [72]}, which introduces the potential of increasing the risk of disease transmission. These high-risk regions can be targeted for elevated disease monitoring to ensure containment ^[47]. In contrast, areas that act as natural barriers, such as rivers, would potentially protect a population by reducing the frequency of new disease introductions, which could facilitate disease eradication in these areas ^[92].

At a local scale, disease transmission is highly influenced by the social behavior of susceptible individuals ^[19]. Individual-based genetic analyses can be used to understand the social dynamics and kinship ^[90]. By using fine-scale genetic techniques, it can be determined whether individuals are spatially proximate, and at what distance these relationships decay ^[39].

Humans have direct and indirect impacts on the demographic and social structure of wildlife populations ^[96]. Alteration of habitats, by human action, can affect home-range size, dispersal, and spatial structure of a population ^[52]. These impacts can also shape the genetic characteristics of a wildlife population ^[55]. When dispersal is disrupted, habitats become fragmented and the spatial dispersion of individuals changes, which in turn affects genetic diversity and structure ^[52].

Harvesting of wildlife also directly impacts a populations abundance and distribution, sex ratios, and age structure ^[58]; illegal harvest of wildlife multiplies human impacts on

wildlife populations. The severity of wildlife crime is difficult to accurately assess as offenses go undetected. Poaching can occur in remote and isolated areas with limited monitoring. The advancement of forensic science is necessary in combating these illegal activities given their high estimated frequency and its inherent threat to the species survival ^[64]. Applicable forensic methods to the enforcement of wildlife legislation largely focus on the use of DNA analysis to identify species and potentially link a sample collected at a crime scene to a particular individual ^[49].

Successful prosecution of wildlife-related crime relies on the individual identification of recovered samples ^[54]. For example, it may be necessary to demonstrate that tissue, blood, or bone has originated from a specific individual in poaching cases. DNA profiling techniques can provide crucial evidence to wildlife crime investigation ^[64]. Adequate recovery of DNA from biological evidence is the most critical stage of any forensic investigation ^[49]. Conservation geneticists have developed techniques to extract DNA to allow for genetic information, such as microsatellite genotypes, to be recovered from almost any biological matter, producing a unique DNA fingerprint ^[80].

Microsatellites are short, highly repetitive sequences that occur in 2-5 base pair repeats ^[49]. Microsatellites are known to be more polymorphic than other genetic markers due to their high mutation rates ^{[5] [7]}. This makes microsatellites highly useful genetic markers when studying wildlife populations; especially in studies focusing on gene flow and dispersal, geographic structure, population history, genetic bottlenecks, and hybridization ^{[6] [9]}.

Anderson et al. (2002) developed a panel of 21 microsatellite loci for use in genetic studies of white-tailed deer (*Odocoileus virginianus*). Though this panel has proven successful in white-tailed deer populations in Oklahoma, it was undetermined if the level of polymorphism would vary in other geographic regions ^[4]. The white-tailed deer has suffered

significant population declines in many areas and potentially experienced demographic and genetic bottlenecks ^[13]. The level of polymorphism of any microsatellite may differ extensively across the range of white-tailed deer, which could reduce microsatellite variation and limit applications that require very low individual ID probability. Using 10 loci from the Anderson et al. (2002) panel, Keeler et al. (2011) evaluated white-tailed deer populations in Monroe County, Pennsylvania. In this study, it was found that 9 out of the 10 loci evaluated would be useful in future studies of white-tailed deer populations in Monroe County and potentially other areas of the state ^[56].

Although microsatellites are conserved within and between species, microsatellites overall effectiveness may vary ^[56]. The number and frequency of alleles can greatly vary between populations of the same species, which may affect the information content of any locus and its overall contribution in the analysis ^{[5] [7] [10]}. It is important to select a panel of loci that provide the largest amount of genetic information, while minimizing the number of microsatellite loci that need to be genotyped. This will ensure that the cost of genetic analysis is minimized while maintaining robust individual identification ^[56].

1.3. History of Wildlife Forensics and the development of Wildlife Forensic Laboratories

Wildlife forensics can be defined as the application of several methodologies of natural and cultural sciences in the courts focused on the regulation of wildlife protection and conservation laws established by regional, national, and international legislation ^[100]. Wildlife crime involves four major categories: (1) illegal taking, or poaching; (2) illegal possession of wildlife; (3) illegal trading, shipping, or moving of wildlife; and, (4) inflicting cruelty to or persecution of wildlife ^[21].

Wildlife crime investigators face a number of complicated factors when applying DNA forensic techniques ^{[49] [78]}. A broad range of target species complicate methods because

it requires development, validation, and reference data for each taxa. Often, the frequency with which a single analysis is employed may be low and therefore it makes it difficult to hire dedicated technicians for these types of cases ^[63]. Finally, the resources available for wildlife forensic work is often lower compared to human forensics, resulting in wildlife DNA forensic services being difficult to maintain ^[20].

A majority of wildlife DNA forensic work typically takes place in academic institutions where scientists with a specific expertise undertakes forensic analysis ^[31]. Academic scientists are essential for the development of new genetic identification techniques and the generation of comparative data ^[79]. However, the potential for forensic genetic approaches to investigate wildlife crime is gradually being realized, resulting in a steadily increasing demand for wildlife DNA forensic services ^[49].

The two core analytical approaches in wildlife DNA forensics are DNA sequencing and fragment analysis. Both methods were developed in the 1980s, and their potential applications to sample identification and legal enforcement has expanded ^[80]. With the support of government resources, these methods were developed during the growth of human DNA forensics and were transferred to accredited forensics laboratory facilities ^[62]. During that time, wildlife DNA forensics remained a highly specialized sub-field, practiced by few scientists. Today, there are a total of 37 wildlife forensic laboratories in the United States, compared to the 400 public crime laboratories across the country (Society for Wildlife Forensic Science). Though there was increasing awareness of the high potential DNA methods had to provide support for wildlife crime investigations, wildlife DNA forensics continued to remain a specialized field; this had a strong influence on the laboratory environment in which wildlife DNA forensic work took place ^[79].

1.4. Primary Goals

The main objective of this thesis is to develop forensic testing methods in order to reduce poaching and support legal hunting in southwestern Pennsylvania. There are two primary goals of this study: (1) develop a molecular forensics panel for identifying white-tailed deer individuals sampled in poaching cases; and, (2) explore the genetic variation and structure of white-tailed deer in southwestern Pennsylvania. By exploring the genetic variation of white-tailed deer, this data will be able to provide insight into the population dynamics of this species that may be important in monitoring CWD and also aid in the development of tools for wildlife management and law enforcement.

Table 1: List of white-tailed deer subspecies and their geographic location in the United States.

Subspecies	Geographic Location
<i>Odocoileus virginianus virginianus</i>	Alabama, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee, West Virginia, Virginia,
<i>Odocoileus virginianus borealis</i>	Connecticut, Delaware, Illinois, Indiana, Iowa, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Wisconsin
<i>Odocoileus virginianus calvium</i>	Florida Keys
<i>Odocoileus virginianus couesi</i>	Arizona and New Mexico
<i>Odocoileus virginianus dacotensis</i>	Colorado, Minnesota, Montana, North Dakota, South Dakota, Wyoming
<i>Odocoileus virginianus hiltonensis</i>	Hilton Head; South Carolina
<i>Odocoileus virginianus leucurus</i>	Oregon and Washington
<i>Odocoileus virginianus macrourus</i>	Arkansas, Iowa, Kansas, Louisiana, Missouri, Nebraska, South Dakota, Texas
<i>Odocoileus virginianus mcilhennyi</i>	Louisiana and Texas
<i>Odocoileus virginianus nigribarbis</i>	Black Beard Island; Georgia
<i>Odocoileus virginianus ochrourus</i>	Northern Rocky Mountains
<i>Odocoileus virginianus osceola</i>	Alabama, midwest Florida, Mississippi
<i>Odocoileus virginianus seminolus</i>	Florida
<i>Odocoileus virginianus taurinsulae</i>	Bulls Island; South Carolina
<i>Odocoileus virginianus texanus</i>	Colorado, Kansas, Oklahoma, Nebraska, New Mexico, Texas, Wyoming
<i>Odocoileus virginianus venatorius</i>	Hunting Island; South Carolina

Chapter Two

Materials and Methods

2.1. Study area and samples

Tissue samples from road-killed and hunter-harvested white-tailed deer in southwestern Pennsylvania were collected and provided by the Pennsylvania Game Commission (PGC). The first set of white-tailed deer samples used for genetic variability analysis were provided by SGW Daniel Puhala from the PGC in Spring 2009; these samples were preserved in jars with 90% ethanol. A second set of samples were provided for this analysis by the PGC from June-October 2018. The samples used for forensic analysis (6 blind controls and 2 cases) were submitted by State Conservation Officers (SCO) from the PGC Southwestern regional office.

2.2. Isolation of DNA

2.2.1. Soft Tissues: DNA from tissue samples was extracted using the DNEasy Blood and Tissue Kit (Qiagen, Valencia, CA). Samples were cut into pieces, weighing 500 mg, and blotted with a kimwipe to remove excess ethanol from the sample. Samples were then placed into a 2.0 mL microcentrifuge tube and 180 µl of Buffer ATL, and 20 µl of proteinase K, were added to the samples and were placed in a thermal shaker at 56°C until the tissue was lysed entirely; lysis time varied between 1-2 days. Once lysed, the samples were vortexed for 15 seconds. After vortexing, 200 µl of Buffer AL was added and thoroughly mixed. This was followed by the addition of 200 µl of ethanol (96-100%) and again by mixing by vortexing. At this stage, white precipitation may form from the addition of Buffer AL and ethanol, but this does not interfere with the results. The mixture was then pipetted into a DNEasy mini spin column and placed in a 2.0 mL collection tube. The tubes were then centrifuged at 8,000

rpm for 1 minute. The flow-through was then discarded, and the DNEasy mini spin column was placed in a new 2 mL collection tube.

Next, 500 μ l of Buffer AW1 was added and centrifuged at 8,000 rpm for 1 minute. The flow-through was then discarded and the DNEasy mini spin column was placed in a new 2 mL collection tube. 500 μ L of Buffer AW2 was added and centrifuged at 14,000 rpm for 3 minutes to dry the DNEasy membrane. It is vital to dry the membrane, or there is a possibility that any remaining product will interfere and contaminate the final eluted product. The flow-through was therefore discarded and placed in a clean 2 mL microcentrifuge tube. An amount of 200 μ L of Buffer AE is pipetted directly onto the DNEasy membrane. The mixture was incubated at room temperature for 1 minute and then centrifuge at 8,000 rpm for 1 minute to elute. The final DNA product was properly labeled and stored at -20°C. To determine the quality of the final DNA product, 4 μ l of DNA was electrophoresed on a 1% agarose gel stained with GelGreen and the quantity of the final DNA product was estimated with 2 μ l of DNA on a NanoDrop.

2.2.2. Bone and Antler: DNA from bone samples was extracted using a user-developed protocol for the Qiagen Blood and Tissue Kit (Qiagen, Valencia, CA). This protocol included the use of EDTA to decalcify the bone samples. Submitted evidence was drilled with a sterile drill bit and collected into a 100 x 15 mm petri dish. 100mg of the powdered bone was transferred into a sterile 50 mL polypropylene tube, 10 mL of 0.5 M EDTA pH 8.0 (Growcells.com, Irvine, CA) was added to decalcify the samples. Tubes were then placed on a rotator and incubated at 37°C for 3 days. After incubation, the tubes were centrifuged at 16,000 rpm for 15 minutes. The supernatant was discarded. The decalcified cells from the powdered bone were then washed with 40 mL of sterile nanopure water to remove ions that have accumulated during decalcification. The tubes were then centrifuged at 16,000 rpm for 15 minutes, the supernatant was then discarded. This washing procedure was

repeated 3 more times. Up to 50 mg of the DNA pellet is placed into a 2 mL microcentrifuge tube. 360 µl of Buffer ATL and 40 µl of proteinase K were added into the tube and mixed by vortexing. Samples were then placed in a thermal shaker and incubated at 56°C until the pellet was lysed entirely; lysis time varied from 1-2 days.

Once lysed, the samples were vortexed for 15 seconds. After vortexing, 400 µl of Buffer AL was added to the sample and was mixed thoroughly by vortexing, this was followed by the addition of 400 µl of ethanol (96-100%) and again mixed by vortexing. At this stage, a white precipitation may form from the addition of Buffer AL and ethanol, but this does not interfere with the results. Up to 650 µl of this mixture was pipetted into a DNEasy mini spin column placed in a 2.0 mL collection tube. The tubes were centrifuged at 8,000 rpm for 1 minute. The flow-through was then discarded, and the collection tube was reused. The remaining 650 µl of the mixture was pipetted into the DNEasy mini spin column placed in a 2.0 mL collection tube. The tubes were then centrifuged at 8,000 rpm for 1 minute. The flow-through was discarded and the DNEasy mini spin column was placed in a new 2.0 mL collection tube. Next, 500 µl of Buffer AW1 was added to the DNEasy mini spin column and centrifuged at 8,000 rpm for 1 minute. The flow-through was then discarded and the DNEasy mini spin column was placed in a new 2.0 mL collection tube.

Next, 500 µl of Buffer AW2 was added to the DNEasy mini spin column and centrifuged at 14,000 rpm for 3 minutes to dry the DNEasy membrane. It is vital to dry the membrane or there is possibility that any remaining product will interfere and contaminate the final eluted product. The flow-through was then discarded and the DNEasy mini spin column was placed in a clean 2.0 mL microcentrifuge tube. 200 µl of Buffer AE was pipetted directly onto the DNEasy membrane. The mixture incubated at room temperature for 1 minute centrifuged at 8,000 rpm for 1 minute to elute. The final DNA product was properly labeled and stored at -20°C. In order to determine the quality of the final DNA product, 4 µl

of DNA was electrophoresed on a 1% agarose gel stained with GelGreen and the quantity of the final DNA product was estimated with a 2 µl of DNA on a NanoDrop.

2.2.3. Processed Meat and Food: DNA from animal food samples was extracted using the DNEasy® Food Mericon Food Kit (Qiagen, Valencia, CA). 200 mg of the frozen food sample was placed in a 2.0 mL microcentrifuge tube, 1.0 mL of Food Lysis Buffer and 2.5 µl of Proteinase K were then added. The solution was vortexed briefly to ensure complete distribution and moistening of the sample material. The tubes are then incubated in a thermal shaker at 60°C; lysis varies from 1-3 days. Once the samples were lysed, the tubes were centrifuged at 2,500 x g for 5 minutes. During centrifugation, 500 µl of chloroform was pipetted in a clean 2.0 mL microcentrifuge tube. Next, 700 µl of the clear supernatant was drawn out and transferred to the 2.0 mL microcentrifuge tube containing chloroform. It is important to not carry over any precipitate, or organic material, from the bottom of the tube when transferring into the chloroform.

The tubes were then vortexed for 15 seconds and centrifuged at 14,000 x g for 15 minutes. If the supernatant is not clear after centrifugation, then centrifuge again for 5 minutes. 350 µl of Buffer PB was then pipetted into a clean 2.0 mL microcentrifuge tube, and 350µl of the upper aqueous layer of the chloroform mixture was added to the tube. Again, mixture was mixed thoroughly. The mixture was then pipetted into a QIAquick spin column, placed into a 2.0 mL collection tube and centrifuged at 17,900 x g for 1 minute. The flow-through was then discarded. The collection tube was reused, and 500 µl of Buffer AW2 was added to the QIAquick spin column. The tubes were centrifuged at 17,900 x g for 1 minute and the flow-through was discarded. The collection tubes were reused and centrifuged again at 17,900 x g for 2 minutes to dry the membrane. Residual ethanol from Buffer AW2 will not be completely removed unless the flow-through was discarded before the additional

centrifugation. The QIAquick spin column was then transferred to a 2.0 mL microcentrifuge tube and 150 µl of Buffer EB was directly pipetted on the QIAquick membrane.

The mixture incubated at room temperature for 1 minute and was centrifuged again at 17, 900 for 1 minute to elute. The final DNA product was properly labeled and stored at -20°C. In order to determine the quality of the final DNA product, 4 µl of DNA was electrophoresed on a 1% agarose gel stained with GelGreen and the quantity of the final DNA product was estimated with 2 µl of DNA on a NanoDrop.

2.3. Amplification of DNA

Samples of extracted DNA were genotyped at 7 microsatellites (BM6506FAM, BM4208NED, BM1225PET, RT7VIC, RT24NED, BM4107PET, and CERVID1FAM) (Table 2) in two separate multiplexes. Multiplex 1 included BM6506, BM4208, BM1225, and RT7, and Multiplex 2 included RT24, BM4107, and CERVID1. Amplifications were done in 10 µl reactions with 1.5 µl of DNA product, 5 µl of Type-it Multiplex PCR Master Mix 2x (Qiagen, Hilden, Germany), 0.1 µl of each of the 7 forward primers for a total of 1.3 µl, 0.1 µl of each of the 7 corresponding reverse primers for a total of 1.3 µl, and 2.7 µl of sterile PCR grade water. PCR was set up as follows: a denaturation step at 95°C for 5 minutes, followed by 40 cycles of a 95°C denaturing for 30 seconds, a 55°C primer annealing step for 90 seconds, and 72°C primer extension step for 30 seconds followed by an elongation step at 60°C for 45 minutes followed by an infinite 4°C hold. PCR amplicons were then diluted with 120 µl of Milli-Q H₂O and 1.5 µl of PCR amplicons were then pooled into a 96 well plate. Next a mixture of Formamide and GeneScan 500 Liz was made and 8 µl of this mixture was added into each well. GeneScan 500 Liz is a size standard designed for sizing DNA fragments in 35-500 nucleotide ranges. The sizing curve generated from these fragments make 500 liz ideal for a variety of fragment analysis applications, such as

microsatellites (Thermo Fisher Scientific, Waltham, MA). Microsatellite PCR amplicons were fractionated on an ABI 3130 Genetic Analyzer (Applied Biosystems, Forest City, CA) and the raw data was then analyzed, and genotype scored, using GeneMarker® (SoftGenetics, State College, PA).

2.4. Amplification of SRY Gene for Sex Identification

For sex ID, samples were amplified, in triplicate, for the presence of the SRY gene, Y53, that is found on the Y chromosome. Amplifications were done in 10 µl reactions with 1.5 µl of DNA product, 5 µl of Type-it Multiplex PCR Master Mix 2x (Qiagen, Hilden, Germany), 0.20 µl of the forward primer, 0.20 µl of the corresponding reverse primer, and 3.10 µl of sterile PCR grade water. PCR was set up as follows: a denaturation step at 95° C for 5 minutes, followed by 40 cycles of denaturing at 95° C for 30 seconds, a 55° primer annealing step for 90 seconds, and a 72°C primer extension step for 30 seconds followed by an elongation step at 60° C for 45 minutes followed by an infinite 4° C hold. The PCR amplicons are then visualized on a 2% agarose gel stained with GelGreen; positive amplification indicates the male gender, whereas, zero amplification indicates the female gender.

2.5. Amplification of D-Loop Region for Species Identification

For species ID, samples were amplified, in triplicate, at the D-loop region. Mitochondrial DNA (mtDNA) is often favored as a genetic marker for species identification of wildlife because mtDNA is easier to type from highly processed and degraded tissue ^[87]. These mtDNA markers have been successfully applied in the identification of wildlife for forensic cases ^[84]. Amplifications were done in 10 µl reactions with 3.0 µl of DNA product, 10.0 µl of Type-it Multiplex PCR Master Mix 2x (Qiagen, Hilden, Germany), 0.40 µl of the

forward primer, 0.40 of the corresponding reverse primer, and 6.20 μ l of sterile PCR grade water. PCR was set up as follows: a denaturation step at 95° C for 5 minutes, followed by 40 cycles of denaturing at 95° C for 30 seconds, a 55° primer annealing step for 90 seconds, and a 72°C primer extension step for 30 seconds followed by an elongation step at 60° C for 45 minutes followed by an infinite 4° C hold. After amplification, PCR amplicons were cleaned by using an UltraClean PCR Clean-Up Kit.

2.6. UltraClean PCR Clean-Up for Species Identification Sequencing

Upon opening the UltraClean PCR Clean-Up Kit, the SpinBind bottle was shaken to mix the solution. Next, 5 volumes of the SpinBind was added to the PCR reaction and mixed by pipetting. The PCR/SpinBind mixture was transferred to a Spin Filter unit and centrifuged for 30 seconds at 10,000 x g (13,000 rpm). After centrifugation, the Spin Filter basket was removed from the tube, and the liquid flow-through was discarded from the tube by decanting. The same Spin Filter was placed back into the same tube, and 300 μ l of SpinClean buffer was added to the Spin Filter. The tube was centrifuged for 30 seconds at 10,000 x g (13,000 rpm). After centrifugation, the Spin Filter basket was removed from the tube and the flow-through liquid was discarded from the tube by decanting. The Spin Filter basket was replaced into the same tube and centrifuged again for 60 seconds at 10,000 x g (13,000 rpm). After centrifugation, the Spin Filter basket was transferred to a clean 2 mL collection tube, and 50 μ l of nanopure H₂O was added to the center of the white Spin Filter membrane to elute. The tube was then centrifuged for 60 seconds at 10,000 x g (13,000 rpm). After centrifugation, the Spin Filter was discarded, and the purified DNA product was now in a labeled 2mL collection tube and is ready to run through a BigDye sequencing reaction.

2.7. BigDye Sequencing Reaction

Sequencing reactions were done in a 5 µl reaction with 1 µl of clean PCR product, 2 µl of BigDye v 1.1, and 1 µl of 2.0 mM Primer. Sequencing was set up as follows: a denaturation step at 96° C for 1 minute, followed by 25 cycles of a 96°C denaturing for 10 seconds, a 50° C primer annealing for 5 seconds, and 60° C elongation step at 60° C for 4 minutes followed by an infinite 4° C hold. After the sequencing reaction was complete, excess dNPs were removed using an Ethanol/EDTA/Sodium Acetate Precipitation, in the below method.

2.8. Ethanol/EDTA/Sodium Acetate Precipitation

A 2.0 mL microcentrifuge tube was prepared, for each BigDye sequencing reaction, that contained: 2 µl of 3M sodium acetate, pH 5.2 and 2 µl of 125 mM EDTA, pH 8.0. The contents of each BigDye sequencing reaction were then pipetted into the tube containing sodium acetate and EDTA. The tubes were then vortexed briefly. Next, 50 µl of 100% ethanol was added to each tube and then vortexed and spun down briefly. The tubes then incubated at room temperature for 15 minutes. After incubation, the tubes for centrifuged at maximum speed for 20 minutes. After centrifugation, the liquid solution was carefully aspirated from the pellet using a pipette tip. The tubes were then quickly spun down again to remove any residual solution. The DNA pellet was then rinsed by adding 250 µl of 70% ethanol and vortexed briefly. The tubes were then centrifuged for 5 minutes at maximum speed.

After centrifugation, the liquid solution was carefully aspirated away from the DNA pellet and then quickly spun down again to remove any remaining solution. The pellets were then dried on a kimwipe for 10-15 minutes. After drying, 10 µl of Formamide was added to each tube. Sequencing analysis was performed on an ABI 3130 Genetic Analyzer (Applied

Biosystems, Forest City, CA). The raw data was analyzed in MEGA7 and sequences were blasted against the NCBI GenBank database (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD). The sequence identity and E-value of the best hit was used to determine if the sample is from a white-tailed deer.

2.9. Genetic Diversity and Hardy-Weinberg Equilibrium

Estimation of genetic diversity is an essential element of population genetic analyses of wildlife. Within-population indices of genetic diversity include the numbers of different alleles per locus, allele richness, and expected (H_e) and observed heterozygosity (H_o). The measures of heterozygosity are highly correlated, but expected heterozygosity is considered a better estimator of the genetic variability present in a population. Expected heterozygosity is a fundamental measure of genetic variation in a population and describes the proportion of heterozygous genotypes expected under Hardy-Weinberg equilibrium (HWE) ^[40]. Estimates of genetic diversity for each sample were calculated, using GenAlEx v6.5, by measuring the mean observed heterozygosity (H_o), expected heterozygosity (H_e), and probability of identity (PI).

A test of HWE should be carried out as an initial step of population genetic analyses. Under the Hardy-Weinberg principle, frequencies of alleles remain constant in a population in the absence of selection, mutation, migration, and genetic drift. Thus, tests of Hardy-Weinberg equilibrium cross-examine the stability of allele frequencies over time. The Hardy-Weinberg principle examines the effects of a single generation of random mating where genotype frequencies can be predicted from the allele frequencies. HWE is expected for populations in which mating is random, and such a population should show no significant difference between observed and expected heterozygosity. Excessive deviation from HWE indicates violation of one of the assumptions of population genetics analyses through, such

processes as natural selection, non-random mating, mutation, genetic drift, and gene flow. However, significant deviation from HWE can also arise from physical error during genotyping ^[59]. Tests of HWE and its significance were carried out using GenAlEx v6.5.

2.10. Population Structure Analysis

To further examine potential population genetic structure, a Principal Coordinate Analysis (PCoA) was performed using a matrix of codominant genotypic genetic differences. Principle Coordinate Analysis (PCoA) is a multivariate method that allows the detection and plotting of major patterns within a data set ^[82]. PCoA aims to summarize the entire genetic variation among individuals that takes into account both variations: between groups (structure genetic variability) and within groups (random genetic variability) ^[95]. The analysis was conducted using GenAlEx v6.5 to visualize the population structure of white-tailed deer in southwestern Pennsylvania. The procedure in GenAlEx v6.5 is based on an algorithm published by Orloci (1978) ^[81]. In addition, population differentiation within southwestern Pennsylvania populations was also analyzed using F_{st} estimated with the AMOVA method in GenAlEx v6.5.

By using a matrix of squared Euclidian distances computed from individual multilocus phenotypes, AMOVA calculates an F_{st} analogue that estimates variation among regions, among individuals, and within populations. AMOVA gives estimates of population genetic structure from dominant markers concordant with those estimated from co-dominant markers, such as microsatellites ^[50].

Table 2: The microsatellite loci, primer sequence, and annealing temperature for each microsatellite locus used.

Microsatellite Locus	Primer Sequence (5' to 3')	Annealing Temp (°C)	Reference
BM6506	F:GCACGTGGTAAAGAGATGGC R:AGCAACTTGAGCATGGCAC	55	Bishop et al. 1994
BM4208	F:TCAGTACACTGGCCACCATG R:CACTGCATGCTTTTCCAAAC	55	Bishop et al. 1994
BM1225	F:ACCCCTATCACCATGCTCTG R:TTTCTCAACAGAGGTGTCCAC	55	Talbot et al. 1996
RT7	F:ACTTTTCACGGGCACTGGTT R:CCTGTTCTACTCTTCTCTC	55	Wilson et al. 1997
RT24	F:CAGTTTAACCAGTCCTCTGTG R:TGTATCCATCTGGAAGATTCAG	55	Wilson et al. 1997
BM4107	F:AGCCCCTGCTATTGTGTGAG R:ATAGGCTTTGCATTGTTTCAGG	55	Talbot et al. 1996
Cervid1	F:AAATGACAACCCGCTCCAGTATC R:TCCGTGCATCTCAACATGAGTTAG	55	DeWoody et al. 1995

Chapter Three

Results: Genetic Variability of Seven Microsatellite Loci in White-Tailed Deer (*Odocoileus virginianus*) from southwestern Pennsylvania

3.1. Study Areas and Sample Collection

For this analysis, 82 white-tailed deer samples were used. Tissue samples were collected from road-killed samples, and hunter-harvested animals in southwestern Pennsylvania provided by the PGC. A total of 44 hunter-harvested samples were provided by SGW Daniel Puhala from the PGC in Spring 2009; it is unknown what part of the animal the samples are from. A total of 38 road-killed samples were collected by the PGC from June-October 2018. Samples were taken from the ear-tip of the deceased animal.

3.2. Genetic Variation Results

A total of 82 white-tailed deer, comprised of 48 females and 28 males were sampled during the study. There were 6 samples in which the sex was unknown. Samples were representative of 25 townships from 8 counties, throughout southwestern Pennsylvania (Figure 1). Of the 82 samples, 80 samples had reportable alleles at all 7 loci (BM6506FAM, BM4208NED, BM1225, RT7VIC, RT24NED, BM4107PET, CERVID1FAM). The number of alleles per locus ranged from 8 (BM1225) to 18 (BM4208 and Cerivd1) (Table 4). The average observed heterozygosity was 0.722 and the average expected heterozygosity was 0.875 (Table 4). The Probability of Identity (PI) ranged from 0.012 (BM4208) to 0.066 (BM1225). The PI for increasing locus combinations (PI Com.) ranged from 2.9×10^{-2} to 7.7×10^{-12} (Table 4). The test for HWE revealed that three out of the seven loci deviated from HWE expectations, these loci included: BM6506, BM4208, and BM1225. BM6506 was found to have a P-value of < 0.00714286 , whereas BM4208 and BM1225 both had a P-value

of < 0.00014286 . Loci RT7, Cervid1F, RT24, and BM4107 did not deviate from HWE expectations.

In the 2009 samples, there were a total of 44 white-tailed deer samples comprised of 37 females and 13 males. Samples were representative of 16 townships from 2 counties, throughout southwestern Pennsylvania. 42 samples had reportable alleles at all 7 loci (BM6506FAM, BM4208NED, RT7VIC, RT24NED, BM4107PET, CERVID1FAM). The number of alleles per locus ranged from 7 (BM1225) to 16 (BM4208) (Table 5). The average observed heterozygosity was 0.711 and the average expected heterozygosity was 0.850 (Table 5). The PI ranged from 0.017 (BM4208) to 0.064 (RT24). The PI Com ranged from 3.9×10^{-2} to 8.0×10^{-11} (Table 5).

In the 2018 samples, there were a total of 38 white-tailed deer samples comprised of 17 females, 15 males, and 6 samples where the sex was unknown. Samples were representative of 25 townships from 8 counties, throughout southwestern Pennsylvania. 38 samples had reportable alleles at all 7 loci (BM6506FAM, BM4208NED, RT7VIC, RT24NED, BM4107PET, CERVID1FAM). The number of alleles per locus ranged from 8 (BM1225) to 12 (RT7; BM4208; Cervid1) (Table 6). The average observed heterozygosity was 0.733 and the average expected heterozygosity was 0.847 (Table 6). The PI ranged from 0.024 (BM4208) to 0.083 (BM1225). The PI Com ranged from 2.8×10^{-2} to 1.2×10^{-10} (Table 6).

3.3. Species Identification and Sex Identification Results

For sex ID, samples were tested for the presence of the SRY male-determining gene by PCR amplification and visualization on an agarose gel. In Case #2018-0040454, evidence 40545-1 and 40545-2 both tested positive for the SRY, identifying the sex of each sample as male. When tested in Case #2018-0048207, evidence 48207-1 and 48207-2 both tested negative for the SRY, identifying the sex of each sample as female.

For Species ID, a segment of the D-loop was PCR amplified and sequenced. The sequences were then compared to curated reference white-tailed deer sequences in GenBank (National Center for Biotechnology Information NCBI). In Case #2018-0040545, both samples resulted in a positive match for white-tailed deer. The E-value for 40545-1 was 0.0 with a sequence identity of 100% to the reference and the E-value for 40545-2 was 0.0 with a sequence identity of 98.29%. In Case #2018-0048207, both samples resulted in a positive match for white-tailed deer. The E-value for 48207-1 was 0.0 with a sequence identity of 99.3% and the E-value for 48207-2 was 0.0 with a sequence identity of 99.0%. All four evidence samples were found to be highly significant in matching with reference white-tailed deer sequences.

3.4. Population Structure Results

After PCoA analysis of a combination of 2009 and 2018 populations, it appears that many of the deer are very similar and cluster together (Figure 2). This is likely due to most of the samples being collected in Allegheny County. However, the plot displays two additional clusters, one cluster is in the bottom right of the plot that consists of 12 deer samples. The other small cluster can be found in the bottom left of the plot consisting of around 12 deer samples but appears to be more similar to the central group.

Similarly, to the analysis in Figure 2, a PCoA analysis of the 2009 population (Figure 3) displays that many of the deer are very similar to one another and tend to cluster together. As with Figure 2, this is most likely due to most of the samples being collected in Allegheny County. On the plot, there are 6 outliers at the top right. These samples are classified as having a geographic origin from both Allegheny county and Beaver county. In contrast, a PCoA analysis of the 2018 populations (Figure 4) show a cluster of samples from 9 different

counties. This plot suggests that white-tailed experience dispersal between counties in southwestern Pennsylvania.

3.5. F_{st} Results

This analysis was ran with 3 populations by using data from both 2009 and 2018 populations (Figure 5). Initially, the goal was to run an analysis with 4 populations: Northern Allegheny, Southern Allegheny, Northern Westmoreland, and Southern Westmoreland. However, Southern Allegheny only had one representative sample to provide for the analysis. Therefore, the analysis was ran with only 3 representative populations. When comparing the molecular variance within individual white-tailed deer in southwestern Pennsylvania, the F_{st} value from this analysis was 0.024, a statistically low variable but significant with a P-value of 0.001. This data implies that there is dispersal among white-tailed deer populations in southwestern Pennsylvania, while maintaining genetic variation between individuals.

Figure 1. Map of 82 white-tailed deer samples used for genetic analysis. Samples were representative of White-tailed deer populations in southwestern Pennsylvania in both 2009 and 2018.

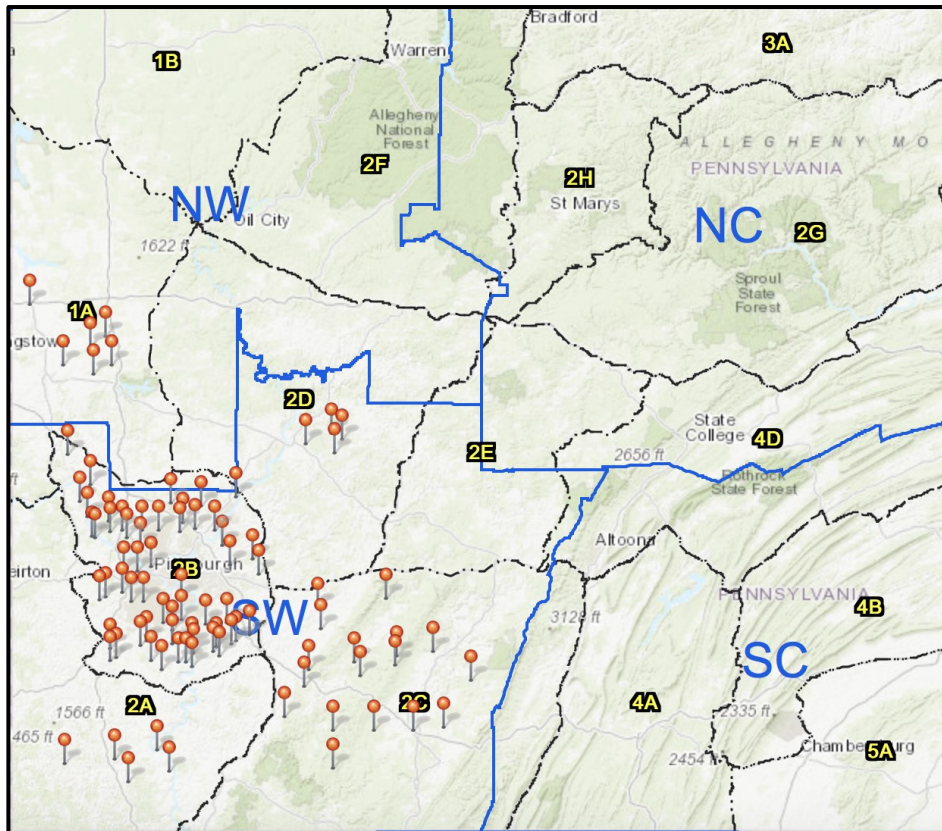


Table 3: Summary of the observed alleles and allelic frequencies for seven microsatellite loci evaluated in Southwestern Pennsylvania white-tailed deer in both 2009 and 2018 populations. All alleles are reported in the number of base pairs.

BM6506		BM4208		BM1225		RT7		RT24		BM4107		Cervid1	
Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
171	0.066	143	0.141	225	0.040	203	0.013	207	0.069	139	0.069	162	0.013
179	0.013	145	0.077	227	0.008	205	0.013	211	0.014	141	0.028	168	0.053
185	0.092	149	0.013	229	0.218	207	0.013	213	0.389	143	0.333	172	0.132
189	0.053	159	0.013	231	0.258	209	0.092	215	0.153	145	0.014	174	0.237
191	0.118	161	0.064	233	0.032	215	0.039	217	0.042	147	0.097	176	0.053
193	0.263	163	0.064	235	0.161	217	0.132	219	0.125	153	0.014	178	0.039
195	0.039	165	0.026	237	0.218	219	0.118	223	0.097	155	0.153	180	0.092
197	0.026	167	0.013	239	0.065	221	0.026	225	0.042	157	0.167	182	0.132
199	0.197	169	0.115			223	0.105	227	0.042	159	0.042	186	0.039
201	0.039	171	0.115			225	0.224	231	0.014	163	0.028	188	0.013
203	0.066	173	0.038			227	0.132	233	0.014	165	0.028	190	0.066
209	0.026	175	0.115			229	0.039			167	0.028	192	0.105
		177	0.090			231	0.053					196	0.026
		179	0.064										
		181	0.026										
		207	0.026										

Table 4: The observed number of alleles, PCR product size range, observed heterozygosity (Ho), expected heterozygosity (He), probability of identity (PI), and PI Combination (PI Com.) for increasing locus combinations for the seven microsatellite loci evaluated in Southwestern Pennsylvania white-tailed deer in both 2009 and 2018 populations.

Microsatellite locus	Observed no. of alleles	Size range Base pairs	Ho	He	PI	PI Com.
BM6506	13	171-209	0.587	0.871	0.029	2.0×10^{-2}
RT7	14	203-233	0.868	0.876	0.026	7.7×10^{-4}
BM4208	18	143-207	0.595	0.918	0.012	9.5×10^{-6}
BM1225	8	225-239	0.677	0.806	0.066	6.3×10^{-7}
Cervid1	18	158-196	0.789	0.902	0.018	1.1×10^{-8}
RT24	15	201-233	0.767	0.881	0.025	2.7×10^{-10}
BM4107	12	139-167	0.771	0.874	0.028	7.7×10^{-12}

Table 5: The observed number of alleles, PCR product size range, observed heterozygosity (Ho), expected heterozygosity (He), probability of identity (PI), and PI Combination (PI Com.) for increasing locus combinations for the seven microsatellite loci evaluated in Southwestern Pennsylvania white-tailed deer populations in 2009.

Microsatellite locus	Observed no. of alleles	Size range Base pairs	Ho	He	PI	PI Com.
BM6506	12	171-209	0.585	0.849	0.039	3.9×10^{-2}
RT7	13	203-231	0.975	0.883	0.025	9.8×10^{-4}
BM4208	16	143-207	0.561	0.904	0.017	1.6×10^{-5}
BM1225	7	225-239	0.581	0.823	0.056	9.2×10^{-7}
Cervid1	13	162-196	0.800	0.874	0.028	2.6×10^{-8}
RT24	11	207-233	0.711	0.789	0.064	1.6×10^{-9}
BM4107	12	139-167	0.763	0.825	0.049	8.0×10^{-11}

Table 6: The observed number of alleles, PCR product size range, observed heterozygosity (Ho), expected heterozygosity (He), probability of identity (PI), and PI Combination (PI Com.) for increasing locus combinations for the seven microsatellite loci evaluated in Southwestern Pennsylvania white-tailed deer populations in 2018.

Microsatellite locus	Observed no. of alleles	Size range Base pairs	Ho	He	PI	PI Com.
BM6506	10	185-203	0.588	0.875	0.028	2.2×10^{-2}
RT7	12	207-233	0.750	0.853	0.034	9.6×10^{-4}
BM4208	12	143-179	0.636	0.883	0.024	2.3×10^{-5}
BM1225.	8	225-239	0.774	0.779	0.083	1.9×10^{-6}
Cervid1	12	158-190	0.778	0.862	0.033	6.5×10^{-8}
RT24	11	201-223	0.829	0.857	0.035	2.3×10^{-9}
BM4107	9	139-163	0.781	0.817	0.054	1.2×10^{-10}

Figure 2. PCoA graph displaying the population structure of Southwestern Pennsylvania White-Tailed Deer Populations in 2009 and 2018.

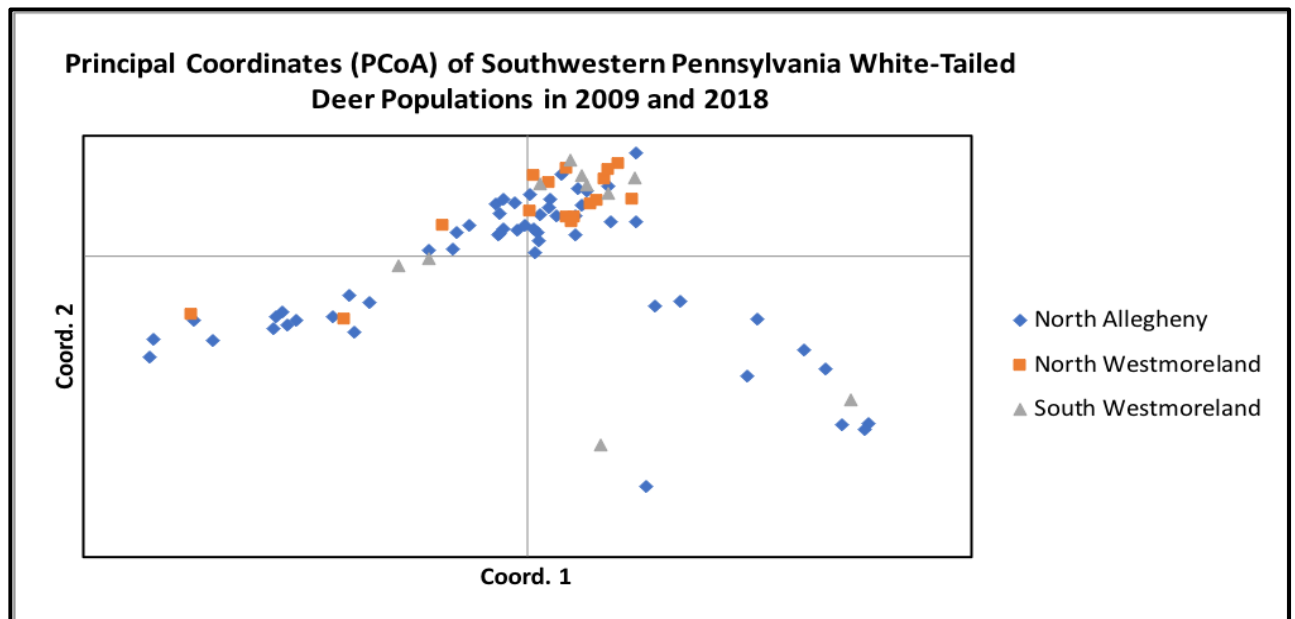


Figure 3. PCoA graph displaying the population structure of Southwestern Pennsylvania White-Tailed Deer Populations in 2009.

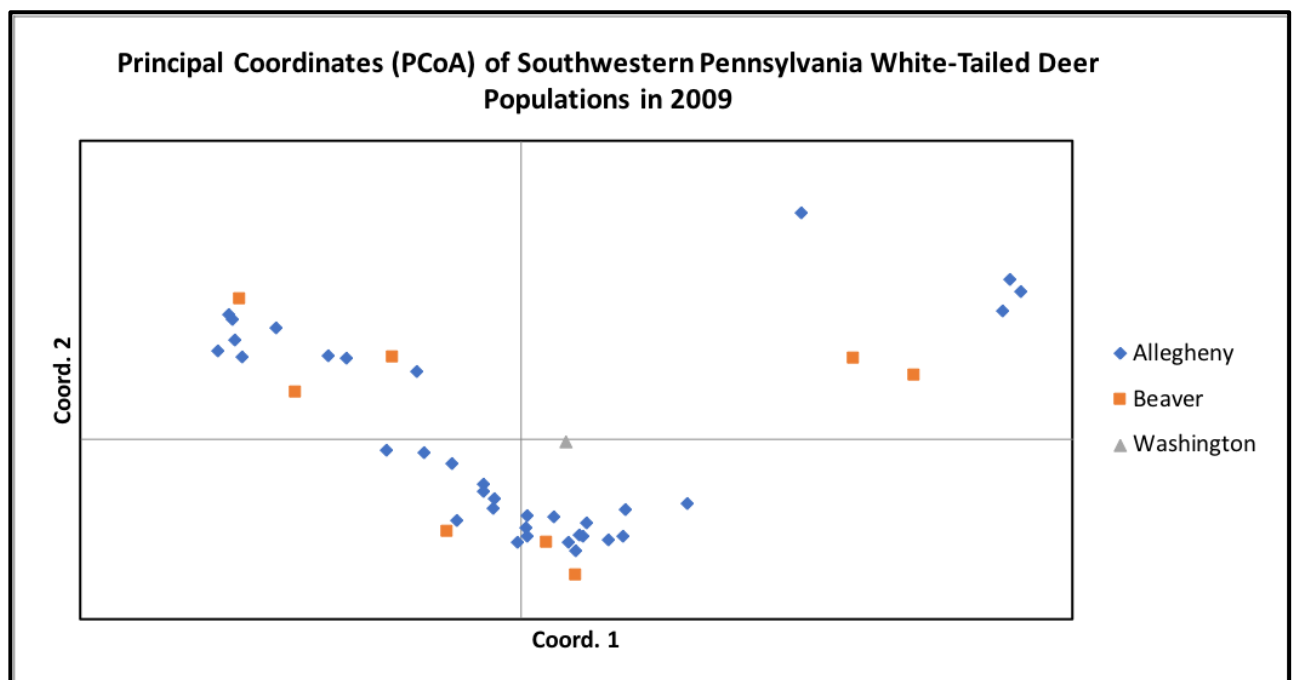


Figure 4. PCoA graph displaying the population structure of Southwestern Pennsylvania White-Tailed Deer Populations in 2018.

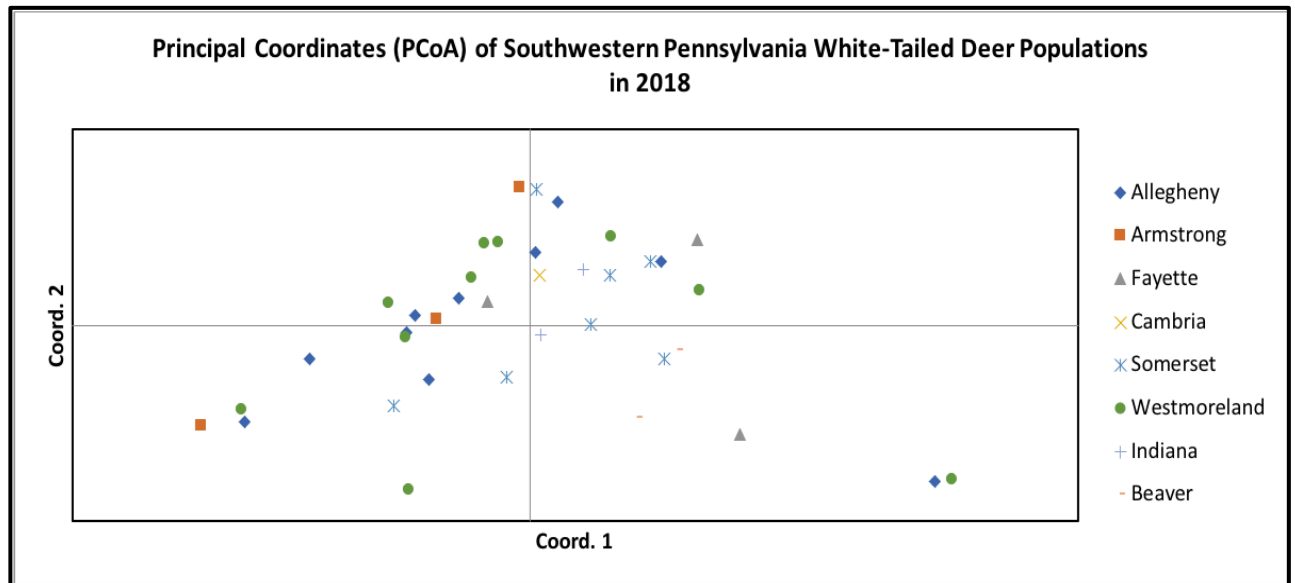
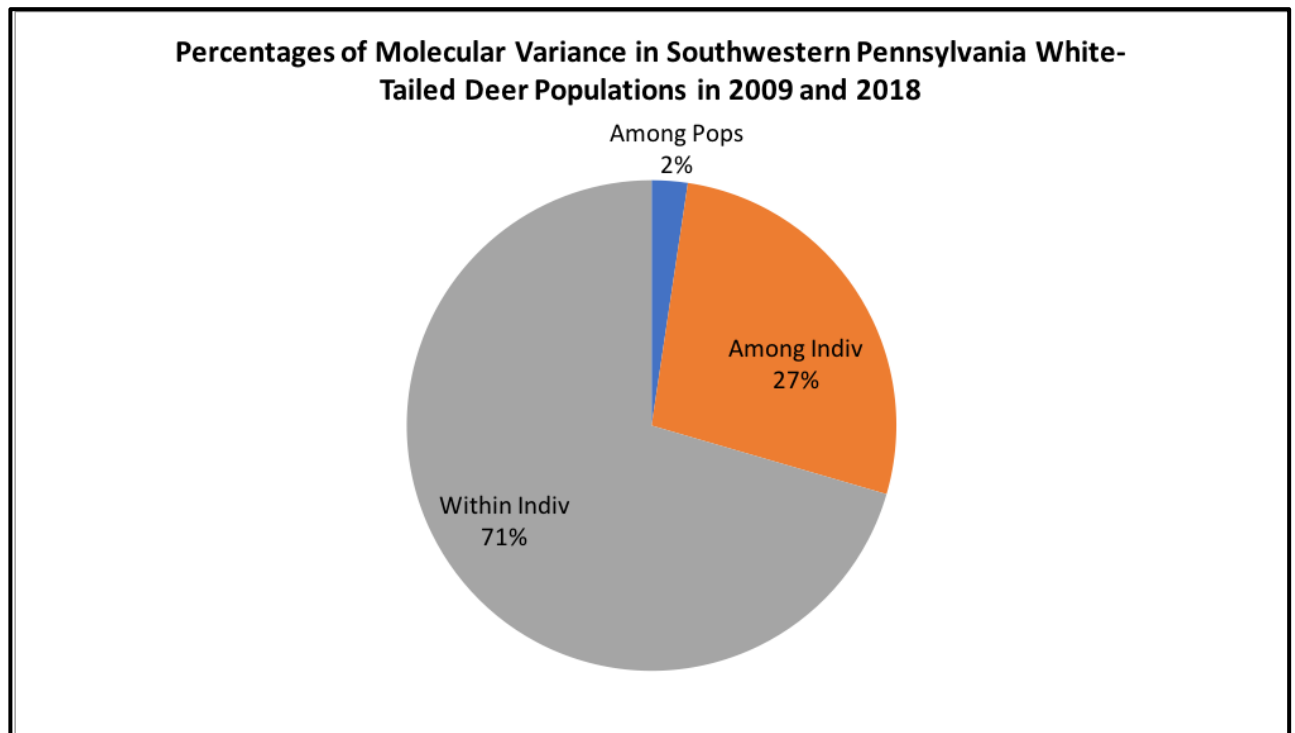


Figure 5. Results of Analysis of Molecular Variance of 3 populations of southwestern Pennsylvania White-tailed Deer in 2009 and 2018 populations.



Chapter Four

Forensic Analysis of PGC Blind Samples, Cases #2018-00040545, and #2018-00048207

4.1. *Pennsylvania Game Commission Blind Samples: June 19, 2018*

To confirm the resolution of our molecular panel and assess the accuracy of forensics testing in the Janecka Genomics Laboratory, the PGC provided the laboratory with a total of 12 blind samples to test the SOPs currently being used. The blind samples were taken from specimen located at the PGC Southwest Regional Office's evidence storage area. Samples were obtained from 6 individual deer, including: 4 individual antler racks and 2 tissue meat samples. Antler rack samples were sampled by the SGW with a hand drill and heat-sterilized drill bit at the base of the antler. The resulting shavings were placed in a 2.0 µl microcentrifuge tube that was then stored in a sterile 50mL polypropylene tube. Samples of meat tissue were taken with a sterilized razor blade and stored in a 50 mL polypropylene tube with 20 mL of 90% ethanol. Each of these 6 samples had a blind duplicate that was taken at the same time by the SGW. The SGW labeled the samples and recorded a key that indicated which duplicate samples came from the same deer. The laboratory was not provided with this information and determined putative matches among all 12 samples based on the microsatellite profile using the developed molecular panel. Samples A through D were powdered bone samples from 4 individual antler racks and samples E-F were tissue samples from 2 different pieces of meat.

On 13 July 2018, DNA was extracted from samples A-D by using SOP No: Ovi_18_002, and on 18 July 2018, DNA was extracted from samples E-F by using SOP No: Ovi_18_001. The final DNA product was labeled and stored at -20° C. To determine the quality of the final DNA product, 4 µl of DNA was electrophoresed on a 1% agarose gel stained with GelGreen. The quantity and quality of the final DNA product was also estimated with a NanoDrop with 2 µl of DNA. Both the DNA yield and DNA quality were significantly low for each sample. After investigation, it was noticed that both the AW1 and AW2 Buffers did not

have the needed 100% ethanol added to each bottle; 100% ethanol was then added to prevent further errors. On 13 August 2018, DNA was extracted from sample sets E-F and E(duplicate)-F(duplicate) by using SOP No: Ovi_18_001. The quality of the final DNA product had shown a higher DNA yield and a higher DNA quality from extraction on 18 July 2018.

On 23 August 2018, sample sets A-D and A(duplicate)-D(duplicate) were decalcified in an EDTA pre-treatment. A total of 0.100 g of each sample were transferred into a sterile 50mL polypropylene tube and 40mL of 0.5M EDTA, pH 8.0 was added to decalcify the samples. The tubes were placed in a heated rotator at 37° C for 4 days. After 4 days, the tubes were centrifuged at 2,000 x g for 15 minutes. After centrifugation, the supernatant was discarded, and the remaining powdered bone was washed with 40mL of sterile nanopure H₂O to remove ions that had accumulated during the decalcification process. The tubes were centrifuged at 2,000 x g for 15 minutes and the supernatant was discarded; this washing procedure was repeated 3 more times. The pellets were then added to labeled 2.0mL microcentrifuge tubes and 360 µl of Buffer ATL and 40 µl of proteinase K was added to the tubes and mixed by vortexing. The tubes were incubated for 24 hours at 56° C in a thermoshaker.

On 24 August 2018, the remainder of the DNA extraction of sample sets A-D and A(duplicate)-D(duplicate) were completed. The final DNA product was labeled and stored at -20°C. In order to determine the quality of the final DNA product, 4 µl of DNA was electrophoresed on a 1% agarose gel stained with GelGreen and the quantity of the final DNA product was estimated with a NanoDrop with 2 µl of DNA. The quality and yield of the final DNA product of each sample was significantly higher than from the extractions done on 13 July 2018.

On 5 September 2018, both sample sets were amplified at 7 microsatellites (BM6506FAM, BM4208NED, BM1225PET, RT7VIC, RT24NED, BM4107PET,

CERVID1FAM,) by using SOP No: Ovi_18_005. The raw data was analyzed, and genotype scored by using GeneMarker®. The microsatellite profile of each sample was analyzed to determine which samples matched one another. In order to match the DNA fingerprints, the electropherogram from each sample were compared which allowed us to determine which samples matched. When these twelve samples were compared to one another, each sample had a genotype that matched to a separate sample (Table 9; Figures 15-20). To add, the probability of identity of these samples was 1 in a billion.

The Blind tests confirmed a high degree of confidence in the developed molecular panel and SOPs being used. The laboratory was now able to transition into accepting white-tailed deer poaching cases from the PGC in the southwest region.

4.2. Case #2018-00040545

On 29 October 2018, evidence for Case #2018-0040545 was picked up at the PGC Southwest Regional Office located at 4820 PA-711, Bolivar, PA; before the evidence was received, a Chain of Custody was signed by both parties. The evidence was transported to the Janecka Genomics Laboratory and evidence was stored as appropriate; Evidence #40545-1, an eight-point antler rack, was stored in a locked cabinet at room temperate. Evidence #400545-2, a mason jar of deer meat, was stored in a locked refrigerator.

On 31 October 2018, powdered bone samples were extracted from #40545-1 by using a hand drill and sterile drill bit. There was a total of four samples extracted from the antler rack: 2 samples from inside of the skull (Inside #1 and Inside #2); and, 2 samples from the spongy bone from both the left and right side of the antler rack (Spongy #1 and Spongy #2).

On 6 November 2018, an EDTA pre-treatment was started on samples, Inside #1; Inside #2; Spongy #1; and, Spongy #2. On 10 November 2018 the remainder of the DNA bone extraction was finished on samples: Inside #1; Inside #2; Spongy #1; and, Spongy #2.

The final DNA product was labeled and stored at -20°C. To determine the quality of the final DNA product, 4 µl of DNA was electrophoresed on a 1% agarose gel stained with GelGreen. The quantity and quality of the final DNA product was estimated with a NanoDrop with 2 µl of DNA. The quality and yield of the final DNA product of each sample was vastly low.

On 10 November 2018, DNA was extracted from evidence #40545-2 by using SOP No: Ovi_18_003. There was a total of three samples extracted from the canned meat: M1; M2; and, M3. The final DNA product was properly labeled and stored at -20°C. The final DNA product was electrophoresed on a 1% agarose gel stained with GelGreen, with 4 µl of DNA product. The quantity and quality of the final product was estimated with a NanoDrop with 2 µl of DNA. The quality and yield of the final DNA product of each sample was vastly low.

Since all samples from Case #2018-0040545 did not yield high-quality DNA product, each sample was cleaned and concentrated with a Zymo Research DNA Clean-Concentrator-S Kit, on 12 November 2018. Samples were added into a 2.0mL microcentrifuge tube and 1,000 µl of DNA binding buffer was added. The mixture was then mixed by vortexing. After vortexing, the mixture was transferred into a Zymo-spin column in a collection tube. The spin columns were centrifuged for 30 seconds and the flow-through was discarded. Next, 200 µl of DNA wash buffer was added to the spin column. The spin columns were then centrifuged again for 30 seconds and the wash step was repeated. After the final wash step, 200 µl of DNA elution buffer was added directly to the spin column membrane and incubated at room temperature for 1 minute. After incubation, the spin columns were transferred into to a 2.0mL microcentrifuge tube and centrifuged for 30 seconds to elute the DNA. The final DNA product was properly labeled and stored at -20°C. In order to determine the quality of the final DNA product, 4 µl of product was electrophoresed on a 1% agarose gel stained with GelGreen. Further, the quantity and quality of the final product was estimated with a

NanoDrop with 2 μ l. The DNA yield was higher than when originally extracted, but the quality of DNA was still low. Since the DNA yield had increased, it was decided to move forward into PCR amplification using Master Mix 1; PCR amplification took place on November 13, 2018.

On 14 November 2018, the PCR amplicons were visualized on a 2% agarose gel stained with GelGreen. The agarose gel showed that the amplicons had successfully amplified, and so it was decided to move forward in the analysis. On 16 November 2018, the PCR amplicons were diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 liz standard and Formamide were added to each well, and the plate was then fractioned on an ABI 3130. The raw data was imported to GeneMarker to analyze and genotyped score, however, the samples did not genotype well and so each sample needed to be re-extracted.

On 29 November 2018, an EDTA pre-treatment was started on samples, Inside #1; Inside #2; Spongy #1; and, Spongy #2. On 4 December 2018, the remainder of the DNA bone extraction was finished on samples: Inside #1; Inside #2; Spongy #1; and, Spongy #2. On 6 December 2018, DNA was extracted from samples M1, M2, and M3 by using SOP No: Ovi_18_003. The final DNA product was properly labeled and stored at -20°C. The quantity and quality of the final DNA product was determined with a Nanodrop with 2 μ l of DNA, and by running the final product on a 1% agarose gel stained with GelGreen with 4 μ l of DNA product. The quality of the final DNA product was significantly higher when compared to both the 6 November 2018 and 10 November 2018 extractions, and so it was decided to move forward into PCR amplification by using both Master Mix 1 and Master Mix 2; PCR amplification took place on 7 December 2018.

On 7 December 2018, the PCR amplicons were visualized on a 2% agarose gel stained with GelGreen with 4 μ l of PCR product. The agarose gel showed that the amplicons had amplified successfully, and so it was decided to move forward in the analysis. On 8

December 2018, the PCR amplicons were diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 liz standard and Formamide were added to each well, and the plate was then fractioned on an ABI 3130 Genetic Analyzer. The raw data was imported to GeneMarker to analyze and genotype score. The genotypes for M1, M2, and M3 were consistent, however, genotypes for samples Inside 1, Inside 2, Spongy 1, and Spongy 2 were not; samples from the antler rack will need to be re-extracted.

On 11 December 2018, powdered bone was extracted from evidence #40545-1 by using a sterile drill and drill bit. There was a total of three samples extracted from the antler rack: 1 sample from inside of the skull (Inside #1); and, 2 samples from the antler base from both the left and right side of the antler rack (Antler Base #1 and Antler Base #2). Each sample was placed into a labeled, sterile 50mL polypropylene tube. On 14 December 2018, an EDTA pre-treatment was started on samples, Inside #1, Antler Base #1, and Antler Base #2. On 17 December 2018, the remainder of the DNA bone extraction was finished on samples Inside #1, Antler Base #1, and Antler Base #2 by using SOP No: Ovi_18_002. The quantity and quality of the final DNA product was determined with a Nanodrop with 2 µl of DNA, and by running the final product on a 1% agarose gel stained with GelGreen with 4 µl of DNA product. The quantity and quality of the final DNA product was significantly higher when compared to the 29 November 2018 extractions, and so it was decided to move forward into PCR amplification by using Master Mix 1; PCR amplification took place on 18 December 2018.

On 18 December 2018, 4 µl of the PCR products were visualized on a 2% agarose gel stained with GelGreen. The agarose gel showed that the amplicons had successfully amplified, and so it was decided to move forward in the analysis. On 19 December 2018, the PCR amplicons were diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 liz standard and Formamide were added to each well, and the plate was then fractioned on an

ABI 3130 Genetic Analyzer. After fragment analysis, the raw data was imported to GeneMarker to analyze and genotype score. The genotypes for Inside #1, Antler Base #1, and Antler Base #2 were consistent.

On 17 January 2019, samples Spongy #1, Spongy #2, M1, Inside #1 (from the 11 December 2018 sample extraction), Antler Base #1, and Antler Base #2 were PCR amplified with Master Mix 2. A total of 4 µl of PCR product was visualized on a 2% agarose gel stained with GelGreen. The agarose gel showed that the amplicons had amplified, and so it was decided to move forward in the analysis. On 19 January 2019, the PCR amplicons were diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 bp standard and Formamide were added to each well, and the plate was then fractionated on an ABI 3130 Genetic Analyzer. The raw data was imported to GeneMarker to analyze and genotype score. The microsatellite profile of samples Inside #1, Antler Base #1, Antler Base #2, and M1 were the most consistent of the samples and so were used for genotype matching. When the genotypes of these four samples were compared to one another, samples Inside #1, Antler Base #1, and Antler Base #2 had genotypes that matched one another, however, the genotype of M1 did not match these samples (Table 7). Therefore, it was ruled that evidence 40545-1 and 40545-2 were not from the same individual. To add, the probability of identity of these samples was one in 1.2 billion.

On 25 January 2019, samples Spongy #1, Spongy #2, M1, Inside #1, Antler Base #1, and Antler Base #2 went through sex ID and species ID analysis. Sex ID analysis follows SOP No: Ovi_18_006 and Species ID analysis follows SOP No: Ovi_18_007.

4.3. Case #2018-00048207

On 17 November 2018, samples for Case #2018-0048207 were received by State Game Warden Brian Singer at the Somerset Bear Check-In Station; before the evidence was

received a Chain of Custody was signed by both parties. The evidence was placed into a locked vehicle and were transported to the Janecka Laboratory later that night. The evidence was removed from the envelope and evaluated. Each evidence sample was stored in a tissue cassette, and so evidence 48207-1 and 48207-2 were both stored in a locked freezer at -20° C.

On 17 December 2018, DNA was extracted from evidence #48207-1 and #48207-2 by using two different SOPs; SOP No: Ovi_18_001 and SOP No: Ovi_18_003. SOP No: Ovi_18_001 was used for samples 48207(1) and 48207(2), while SOP No: Ovi_18_003 was used for 48207(2)-FL. The DNA extractions were finished on 19 December 2018. The quantity and quality of the final DNA product was determined with a Nanodrop with 2 µl of DNA, and by running the final product on a 1% agarose gel stained with GelGreen with 4 µl of DNA product. The quantity and quality of the final DNA product were substantial, and so it was decided to move forward into PCR amplification by using Master Mix 1; PCR amplification took place on 19 December 2018.

On 19 December 2018, the PCR amplicons were visualized on a 2% agarose gel stained with GelGreen. The agarose gel showed that the amplicons had amplified successfully, and so it was decided to move forward in the analysis. On 20 December 2018, the PCR amplicons were diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 bp standard and Formamide were added to each well, and the plate was then fractionated on an ABI 3130 Genetic Analyzer. The raw data was imported to GeneMarker where it was analyzed, and genotype scored. The genotypes for 48207(2) and 48207(2)-FL were consistent, however, 48207(1) was not; 48207(1) will need to be re-extracted.

On 9 January 2019, DNA was extracted from evidence #48207-1 using SOP No: Ovi_18_001. To determine the quality of the final DNA product, DNA was electrophoresed on a 1% agarose gel stained with GelGreen and the quantity of the final DNA product was

estimated with a NanoDrop. The quantity and quality of the final DNA product was substantial.

On 4 February 2019, samples 48207(1) (from 9 January 2019 extraction), 48207(2), and 48207(2)-FL went through sex ID and species ID analysis. Sex ID analysis follows SOP No: Ovi_18_006 and Species ID analysis follows SOP No: Ovi_18_007.

On 25 February 2019, samples 48207(1) (from 9 January extraction), 48207(2), and 48207(2)-FL were PCR amplified with Master Mix 1 and Master Mix 2. The PCR amplicons were visualized on a 2% agarose gel stained with GelGreen, with 4 µl of PCR product. The agarose gel showed that the amplicons had amplified successfully, and so it was decided to move forward in the analysis. The PCR amplicons were then diluted with Milli-Q H₂O and pooled into a 96 well plate. A 500 bp standard and Formamide were added to each well, and the plate was then fractionated on an ABI 3130 Genetic Analyzer. The raw data was imported to GeneMarker where it was analyzed, and genotype scored. The genotypes for all samples were consistent.

The microsatellite profiles of samples 48207(1) re-extraction, 48207(2), and 48207(2) were used for genotype matching. When the genotypes of these three samples were compared to one another, the electropherograms of all three samples genotypes had an exact match to one another (Table 8). Therefore, it was ruled that evidence 48207-1 and 48207-2 were from the same individual. To add, the probability of identity of these samples was less than one in 1.2 billion.

4.4. Conclusions

A total of 12 blind controls were correctly identified. These methods were successfully used to analyze poaching cases for the PA Game Commission. All protocols were written into easy to follow SOPs and are available in Appendix 1. Additionally, the

Chain of Custody and Case Submission Forms were also developed and written prior to accepting these two cases, as well. These forms are available in Appendix 1. The 7 microsatellite primers used in these analyses were chosen by their effectiveness in previous studies of white-tailed deer and by how well the amplicons worked with the reference sample database compiled of white-tailed deer samples from 2009 and 2018 populations in southwestern Pennsylvania. The success of the two poaching cases discussed in this thesis provides a high degree of confidence to continue to provide this service for the PGC, and the potential to work with other state agencies in the future.

Table 7. Table of genotypes of samples from Case #2018-00040545.

	BM6506	BM4208	BM1225	RT7	RT24	BM4107	Cervid1
40545-1	183/191	173/175	229/231	223/227	213/215	145/163	170/180
40545-2	191/191	147/163	231/235	211/225	-	137/145	184/190
Positive Control	191/197	169/177	231/235	219/227	211/217	141/153	178/178

Table 8. Table of genotypes of samples from Case #2018-00048207.

	BM6506	BM4208	BM1225	RT7	RT24	BM4107	Cervid1
48207-1	189/197	159/179	229/235	219/223	209/217	153/155	178/194
48207-2	189/197	159/179	229/235	219/223	209/217	153/155	178/194
Positive Control	187/197	141/147	229/235	221/227	211/227	141/153	176/180

Table 9. Table of genotypes of samples from PGC Blind Tests.

	BM6506	BM4208	RT7
Blind A	191/197	-	-
Blind B	189/195	-	-
Blind C	183/183	159/171	225/231
Blind D	197/197	147/163	219/227
Blind E	201/201	159/159	219/227
Blind F	-	159/163	209/229
Blind A (dup.)	191/197	-	-
Blind B(dup.)	183/183	159/171	225/231
Blind C(dup.)	197/197	147/163	219/227
Blind D(dup.)	189/195	-	-
Blind E(dup.)	-	159/163	209/229
Blind F(dup.)	201/201	159/159	219/227

Table 10. The quality and quantity of the final DNA products extracted during the PGC Blind Tests.

	ng/ μ l	A260/A280
18 July 2018		
Blind A	6.3	1.51
Blind B	10.0	1.75
Blind C	4.4	1.82
Blind D	5.2	1.47
Blind E	1.2	1.79
Blind F	7.1	1.38
13 August 2018		
Blind E	14.0	1.92
Blind F	19.8	1.87
Blind E (dup.)	21.1	1.73
Blind F (dup.)	16.0	1.83
24 August 2018		
Blind A	0.7	1.72
Blind B	5.9	1.85
Blind C	3.8	1.84
Blind D	0.5	1.75
Blind E	5.2	1.80
Blind F	4.8	1.85
Blind A (dup.)	6.1	1.46
Blind B (dup.)	4.6	1.75
Blind C (dup.)	8.3	1.41
Blind D (dup.)	37.3	1.45
Blind E (dup.)	7.3	1.47
Blind F (dup.)	10.5	1.34

Table 11. The quality and quantity of the final DNA products extracted during Case #2018-00040545.

	ng/ μ l	A260/A280
6 November 2018		
Inside 1	-1.7	0
Inside 2	3.5	0
Spongy 1	-0.5	0
Spongy 2	0.1	0
10 November 2018		
M1	2.4	0
M2	2.0	2.00
M3	3.2	2.00
12 November 2018		
Inside 1	10.9	1.02
Inside 2	17.3	1.21
Tissue	26.8	1.17
Spongy 1	5.5	0.73
Spongy 2	5.8	0.81
M1	6.5	0.84
M2	24.8	1.37
M3	9.7	0.98
6 December 2018		
Inside 1	1.5	1.76
Inside 2	0.7	1.76
Spongy 1	10.0	1.83
Spongy 2	9.2	2.09
M1	0.5	2.09
M2	0.9	2.09
M3	1.5	2.09

Table 12. The quality and quantity of the final DNA products extracted during Case #2018-00048207.

	ng/ μ l	A260/A280
19 December 2018		
48207(1)	215.3	1.85
48207(2)	116.2	1.90
48207(2)-FL	111.1	1.78

Figure 6. Case #2018-00040545. Figure of the quality and quantity of the final DNA product for samples I1, I2, S1, S2 on 16 November 2018.

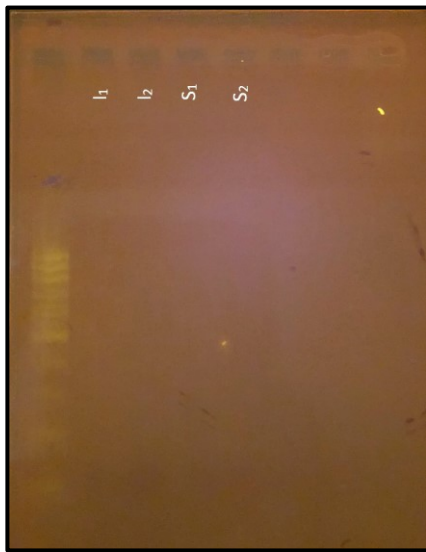


Figure 7. Case #2018-00040545. Figure of PCR amplification, using Panel 1, with samples I1, I2, S1, S2, Tissue, M1, M2, and M3 on 7 December 2018.

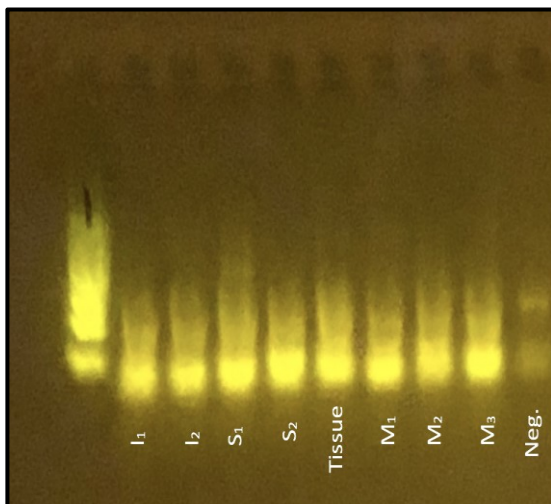


Figure 8. Case #2018-00040545. Figure of PCR amplification, using Panel 2, with samples I₁, I₂, S₁, S₂, Tissue, M₁, M₂, and M₃ on 7 December 2018.

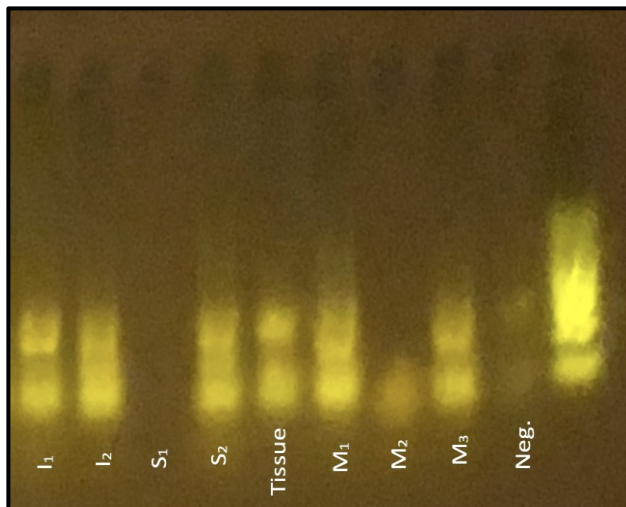


Figure 9. Case #2018-00040545. Figure of PCR amplification, using Panel 1, with samples I₁, I₂, Tissue, S₁, S₂, M₁, M₂, M₃, and Blind B post Zymo Clean Kit on 12 November 2018.

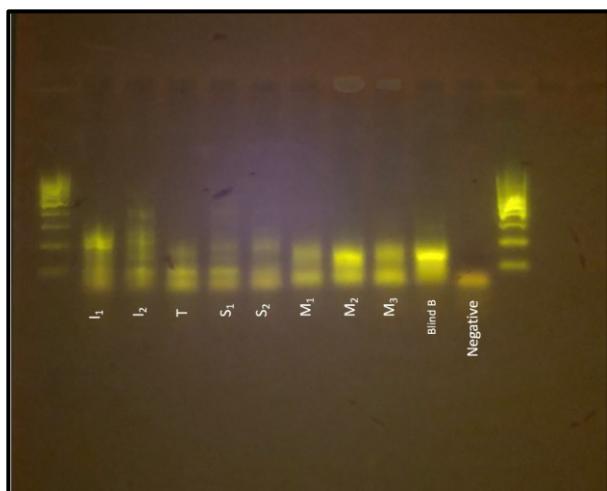


Figure 10. Case #2018-00040545. Figure of PCR amplification at SRY gene for Sex ID and the D-loop region for Species ID with samples Spongy #1, Spongy #2, M₁, Inside #1, AB #1, AB #2, Ovi0085, and Ovi0086 on 25 January 2019.

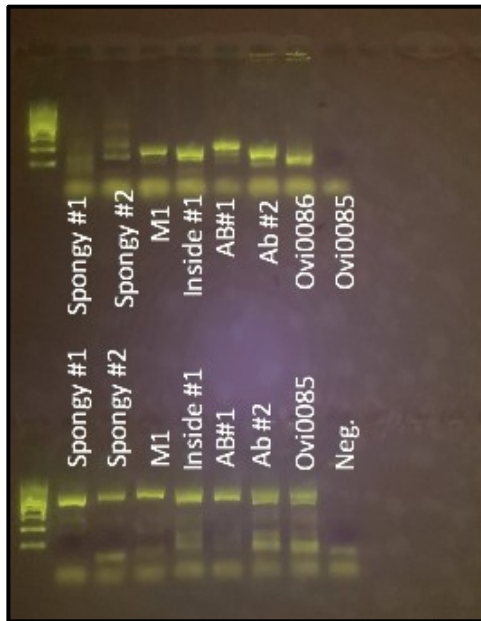


Figure 11. Case #2018-00040545. Figure of PCR amplification, using Panel 1, with samples Spongy #1, Spongy #2, Inside #1, M₁, AB#1, AB#2, and Ovi0085 on 18 December 2018.

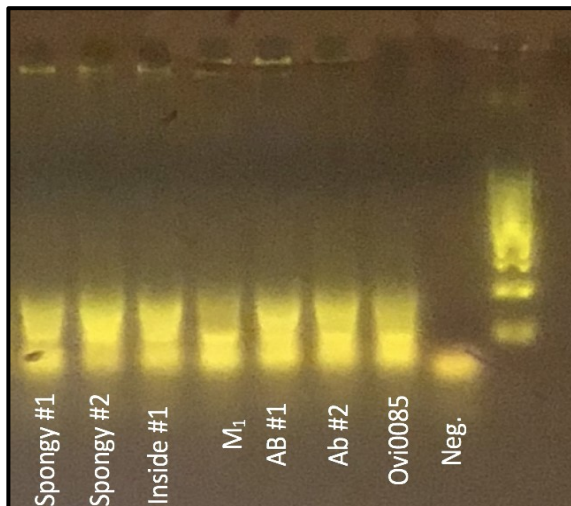


Figure 12. Case #2018-00040545. Figure of PCR amplification, using Panel 2, with samples Spongy #1, Spongy #2, Inside #1, M₁, AB #1, AB #2, and Ovi0085 on 17 January 2019.

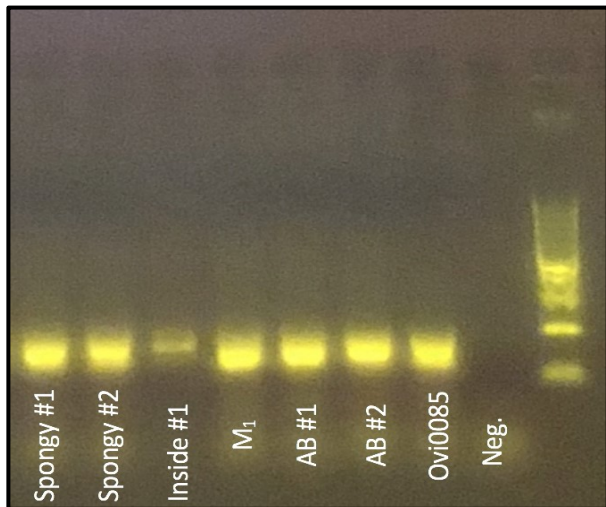
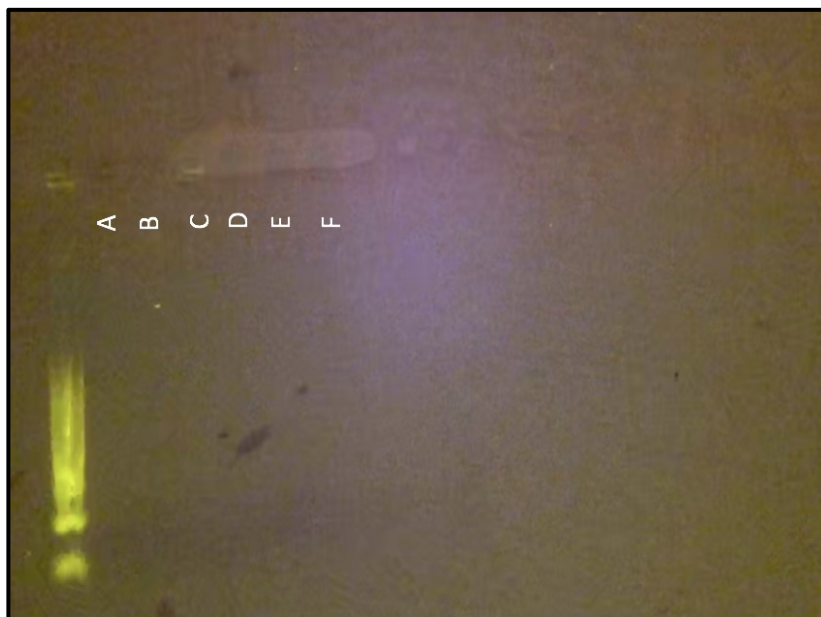


Figure 13. PGC Blind Samples. Figure of the quality and quantity of the final DNA product For samples A, B, C, D, E, and F on 18 July 2018.



E_2
 F_3
 Ovi0043
 Ovi0044
 Ovi0045
 Negative

A
 B
 C
 D
 E
 F
 A_2
 B_2
 C_2
 D_2
 E_2
 F_2
 Ovi0043
 Ovi0044
 Ovi0045
 Negative
 A
 B
 C
 D
 E
 F
 A_2
 B_2
 C_2
 D_2

The figure displays two mass spectra plots side-by-side, comparing the fragmentation patterns of BM6506-FAM and BM6506-FAM-191. The top plot, labeled 'frag_001_BlindA_M1.fsa', shows a base peak at m/z 191 and a significant peak at m/z 197. The bottom plot, labeled 'frag_003_BlindA2_M1.fsa', shows a base peak at m/z 191 and a significant peak at m/z 197. Both plots have an x-axis representing m/z from 160 to 210 and a y-axis representing relative intensity from 0 to 300 (top) or 0 to 400 (bottom). The peaks are labeled with their m/z values, 191 and 197, in green boxes.

Figure 17. Figure of DNA Finger Print Match of C and B duplicate.

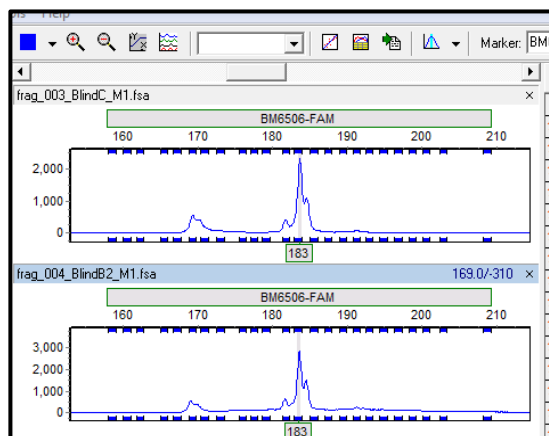


Figure 18. Figure of DNA Finger Print match of D and C duplicate.

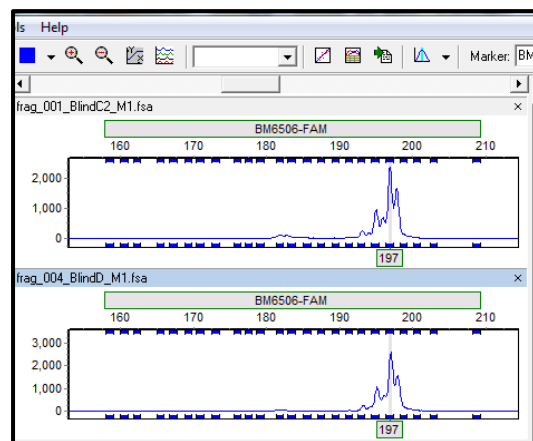


Figure 19. Figure of DNA Finger Print Match of E and F duplicate

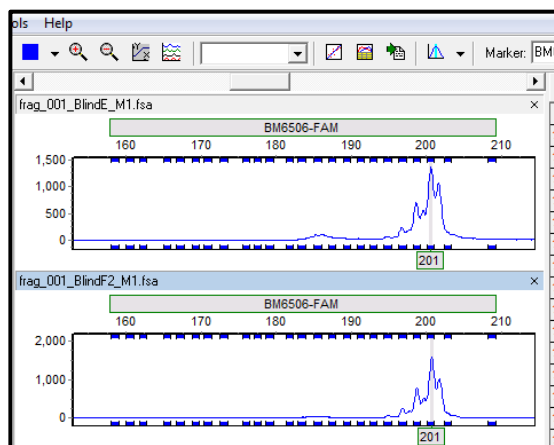
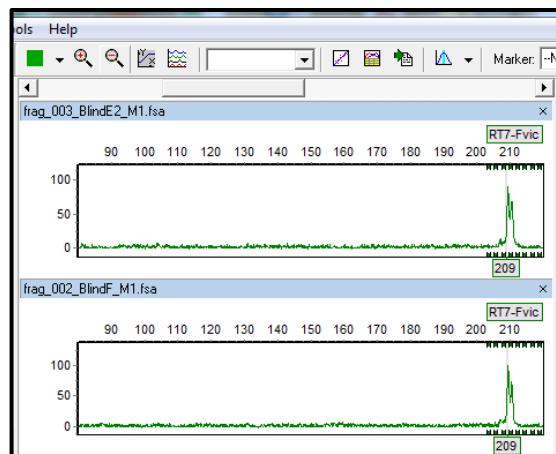


Figure 20. Figure of DNA Finger Print Match of F and E duplicate



Chapter Five

Discussion and Conclusions

5.1. Application of Genetics to White-Tailed Deer in the State of Pennsylvania

The core of conservation genetics is the evaluation of genetic variation within and among populations ^[33]. Evaluating genetic variation provides information for the maintenance of natural levels of genetic diversity ^[33] ^[97]. The evaluation of genetic variation within wild populations highlights conservation and management risks, while also evaluating genetic variation among geographic populations ^[77]. Typically, studies of genetic variation utilize markers that are selectively neutral ^[5], however, these markers do not necessarily correspond to levels of diversity found in genes that are found to impact the fitness of an individual ^[74], proxies of fitness, such as heterozygosity in selectively neutral markers, are important for evaluating the conservation and management status of a population ^[88].

Genetic variation can be used to evaluate how populations within a species have been historically separated over time and may also provide clues to events responsible for a populations' current level of variation ^[26]. Analyzing the genetic variation of white-tailed deer populations is critical for deer management in Pennsylvania. This information allows wildlife managers to determine hunting quotas and management strategies, for each season, in order to maintain healthy populations based on their geographic location ^[38] ^[51]. Poor management practices have the potential to reduce the overall fitness of a population and may also lead into a decline in abundance ^[48].

It is important for wildlife managers to be aware of the genetic variation of any population(s) being managed. Genetic markers offer the opportunity to evaluate the genetic structure of local populations ^[5] ^[7] ^[61]. In addition, these markers can be used to determine the number of breeding individuals and relationships within a herd ^[15]. As the number of

breeding individuals decreases, the level of heterozygosity will decrease ^[52]. Sequentially, this loss of variation has potential to cause a loss in fitness and population sustainability ^[48].

In the early 1900s, deer were abundant in the northern forests of Pennsylvania, but were found to be scarce in urban areas and southern farm areas. However, in present day, the deer abundance between the northern forests and urban and southern areas have reversed. Deer populations are now ample in some of the most developed areas and are less abundant in some of the more forested areas (Pennsylvania Game Commission 2009-2018 Deer Management Plan). This change in preferred habitat has been caused by human development. More heavily developed areas provide an adequate amount of food, ideal living conditions, and reduced predation risks. As development increases, the limitation of accessible land for hunters also increases. Today, deer are well-fed and reproducing at high rates, with a reduced risk of being killed by hunters. However, deer residing in forested areas are found to be more susceptible to predation and hunters, have fewer accessible food sources, and reproduce at lower rates (Pennsylvania Gam Commission 2009-2018 Deer Management Plan). By occupying more ideal habitats, white-tailed deer can attain their necessary requirements in smaller areas whereas deer residing in areas with fewer resources are forced to travel further distances to fund suitable food and habitat ^[65].

The PGC's management goals for white-tailed deer include: (1) manage deer for a healthy and sustainable herd; (2) manage deer-human conflicts at levels considered safe and unacceptable to Pennsylvanian's; (3) manage deer impacts for healthy and sustainable forest habitat; (4) manage deer to provide recreational opportunities; and (5) improve the public's knowledge and understanding of deer and the deer management program (Pennsylvania Game Commission 2009-2018 Deer Management Plan).

The broad goal of this thesis was to develop forensic testing methods in order to aid in the development of tools for wildlife management and law enforcement in the state of

Pennsylvania; this was done through the development of a molecular panel. This panel was also developed to determine the genetic variability within white-tailed deer populations in southwestern Pennsylvania. This data will contribute to future studies in white-tailed deer genetic variation. The specific goals and outcomes of this project have been discussed in previous chapters. In brief, a molecular panel was developed in order to characterize the genetic variability in white-tailed deer and assess their genetic structure. The development of this molecular panel also allowed for the forensic analysis of PGC white-tailed deer poaching cases.

Keeler et al. (2011) evaluated 10 microsatellite loci within white-tailed deer populations in Monroe County, PA. It was found that 8 of the 10 microsatellite primer pairs were successful in allele amplification and could be useful in future studies of white-tailed deer in Monroe County, and other areas of Pennsylvania ^[56]. The seven loci evaluated in this study were selected using previously published data from Keeler et al. (2011), and Anderson et al. (2002). Our selections were based on the following criteria: (1) number of loci; (2) genetic information content based on heterozygosity; and (3) size range of loci. These criteria were used to maximize the genetic information that would be gained from the panel while also limiting the cost of the analysis through limiting the number of runs on the Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Forest City, CA) and for the development of multiplexed PCR reactions used for the analysis.

In this thesis, all seven microsatellite primer pairs were effective at amplifying alleles in all evaluated samples, however, when observed and expected heterozygosity's were compared, HWE revealed that three out of the seven loci deviated from HWE expectations. These loci included: BM6506, BM4208, and BM1225. BM6506 was found to have a P-value of < 0.00714286 , whereas BM4208 and BM1225 both had a P-value of < 0.00014286 . A potential cause of this deviation may have been caused by a small number of reference

individuals that could potentially be homozygous for a rare allele, additionally, smaller sample sizes and low genotyping error rates may also cause deviation. Allelic dropout during PCR may also falsely read as a homozygote, when it should be read as a heterozygote, which will also affect HWE.

The probability of identity (PI) was used to evaluate the overall effectiveness of using microsatellite loci for individual identification. Probability of identity is the probability that two individuals drawn at random from a population will have the same genotype ^[99]. It is also used to provide statistical confidence for identifying individuals ^[98] and quantifying the genetic diversity in a population ^[85]. PI is calculated by squaring the match probability for each genotype summed over all possible genotypes ^[99]; this compares two individuals drawn at random from a population. PI can be estimated for differing numbers of loci without having forensic genotypes available, which makes PI highly useful in studies that require individual identification ^[99].

In Keeler et al. (2011), 8 of the 10 microsatellite primers were effective at amplifying in all Monroe County deer evaluated and the primer for BM1225 was effective for 33 of the deer; BM1225 failed in the remaining samples. Based on these results, the primers evaluated were determined useful for future studies in white-tailed in Monroe County and potentially in other areas of Pennsylvania. The primer BM4208 was only effective for 5 of the deer evaluated in the study, however, BM4208 was effective at amplifying alleles within Oklahoma white-tailed deer populations in the Anderson et al. (2002) study. The microsatellite loci BM6506, BM4208, BM1225, RT7, RT24, BM4107, and CERVID1 were evaluated in both Oklahoma and Monroe County, PA deer and the number of alleles, allelic frequencies and statistical values calculated for these numbers were consistent with this thesis ^[4] ^[56]. This demonstrates the effectiveness of microsatellites that can occur between populations of the same species.

Our PI values indicate that the microsatellite panel used in this thesis is proven to be effective for forensic analysis of white-tailed deer in southwestern Pennsylvania. Species identification may be used in cases of poaching to identify trace evidence in the field or from a suspects' possessions. This panel has demonstrated its usefulness for studies on populations in western Pennsylvania and neighboring areas when predicting expected genotype frequency and indicating gene flow between populations. These microsatellite loci allow for efficient and cost-effective means of obtaining multiple genotypes from a single individual through a combination of PCR and fragment analysis.

Overall, the results from this thesis recognize high genetic variability among white-tailed deer populations in southwestern Pennsylvania, which likely results from the species natural-history patterns. White-tailed deer underwent an extensive restocking program after their near extirpation in the early 1900s, in addition to other management plans put into action. Following these conservation and management actions, white-tailed deer were able to naturally repopulate at a rapid rate. It is from these events that leads to believe why the genetics of white-tailed deer in this area is highly variable. In addition, these events suggest that populations have experienced genetic bottlenecks that are consistent with their demographic history ^[26]. Typically, female white-tailed deer give birth to their first offspring at 2 years of age ^[43] ^[68], meaning that there is a minimum generation time of 2 years that can be assumed ^[27]. If population recovery in Pennsylvania began in the early 1900s, it is estimated that 35 generations have elapsed, which approaches the estimated detectable limit for mode-shifted allele frequencies ^[27]. Though Pennsylvania populations underwent a decrease in population size, there are no genetic bottlenecks present. This may have been caused by historically effective population sizes that may have been sufficient enough to where the effects of genetic bottlenecks were not detectable despite known demographic bottlenecks ^[27]. However, it is possible that these uneven bottleneck signatures could involve

an admixture of individuals from genetically distinct populations ^[42]. Admixture can increase the occurrence of rare alleles without significantly affecting heterozygosity ^[23].

Data in this thesis highlights the importance of understanding population genetic structure and variation as it relates to identifying population dynamics among white-tailed deer. Despite the evidence of past demographic bottlenecks, restored Pennsylvania deer populations have shown high genetic diversity in their populations. The genetic diversity observed among these populations suggests that they have undergone a rapid expansion and that habitat continuity has played an important role in the genetic diversity and variation of white-tailed deer individuals; habitat continuity promotes gene flow and reduces genetic drift ^[56]. White-tailed deer in Pennsylvania quickly reached large post-recovery population sizes which have been found to maintain more variation. Any losses of heterozygosity are found to occur over a short period of time, which may explain why white-tailed deer in Pennsylvania have maintained their high levels of genetic variation ^[42]. Unlike other restored species, white-tailed deer have access to habitat distribution with a virtual absence of geographical barriers ^[27], and so, these factors may be important in homogenizing the levels of genetic variation as bottleneck signatures can quickly be erased in the presence of low levels of immigration ^[56].

Additionally, behavioral factors may have also contributed to genetic diversity being maintained in white-tailed deer. For example, the reproductive ecology of white-tailed deer differs from that of other large mammal species. White-tailed deer have a tending-bond mating system ^[46] which could potentially decrease the variance in male reproductive success relative to other mating systems ^[93]. Females of this species are promiscuous and the frequency of multiple paternity in a single litter increases, dependent on the situation ^[27]. Additionally, white-tailed deer are more r-selected, meaning that populations of this species are governed by their maximum reproductive capacity, than many other large mammals. This

trait is represented by the regular production of twin offspring and an earlier age of first reproduction ^[35]. The combination of these factors may result in a high effective population size for white-tailed deer when compared to other species of large mammals ^[27].

The white-tailed deer recovery program was highly successful in restoring deer to their former range in Pennsylvania while avoiding the loss of genetic diversity. Despite the presence of some historic patterns of genetic variation, most genetic diversity in present-day populations is broken up within populations with little or no higher-level structuring ^[26]. This is to be expected in populations that have experienced bottlenecks, unequal population sizes and genetic drift ^[16] ^[41]. The relocation of large numbers of deer into Pennsylvania territory likely increased the chance of success for individual translocations and contributed further to the fine-scale genetic structuring ^[42]. Despite the potential gene flow, in a highly vagile species, the effect of these translocations is still detectable in many populations which indicates that higher-order structure is not reforming ^[26]. This is consistent with evidence that social factors contribute to substructuring on a microgeographical scale in white-tailed deer populations ^[86]. Therefore, patterns of genetic distance and fine-scale genetic structure will continue to be a persistent feature of Pennsylvania deer populations. The data in this thesis provides valuable information to aid in long term management strategies, which aim to preserve genetic variability in white-tailed deer in the state of Pennsylvania.

5.2. Application of Molecular Panel to Wildlife DNA Forensics

DNA technologies have grown to allow the ability to solve wildlife crime. In this thesis, these technologies have been applied in context of two case studies involving white-tailed deer. As such, this thesis has made contributions both to the discipline of wildlife forensics and to the application of the population genetics of white-tailed deer. Through the analysis of gene flow and dispersal, population genetics allows us to understand both species

and individual identification in wildlife forensics. The application of the developed molecular panel in this thesis was able to contribute to the management of illegal hunting activities in the southwest region of the state of Pennsylvania.

The use of this panel has helped the PGC in utilizing their resources in determining whether an individual should be prosecuted for a wildlife crime. In Case #2018-00040545, the final genotypes of 40545-1 and 40545-2 did not match one another. This shows that our panel was able to determine that the suspected perpetrator in this case had not participated in the act of illegal harvest while their hunting license had been revoked. Having this information, the PGC did not have to waste any resources in further examining the situation. However, in Case #2018-00048207, the genotypes for 48207-1 and 48207-2 were exact matches to one another. This shows that the molecular panel was able to determine that the suspected perpetrator had in fact participated in the act of illegal harvest. By providing the PGC with this information, law enforcement is now able to move forward in prosecuting the appropriate offender. By continuing this service, the use of this molecular panel will continue to aid and grow in the conservation and management of white-tailed deer not only in Pennsylvania, but also in surrounding areas.

5.3. Application of Molecular Panel for CWD Management

White-tailed deer, like most animals, are subject to a variety of diseases and health problems. Some diseases are deer specific, but others can be transmitted to other animals, including humans. CWD is a fatal disease of the nervous system; the disease causes microscopic holes in brain tissue giving it a sponge-like appearance^[57], similar to “mad cow” disease in cattle. The exact mode of transmission of CWD from animal to animal is still unknown, but it is believed to be spread through body fluids, fecal material, or contaminated environments^[75].

By using genetic data, scientists and wildlife managers are able to identify the genetic risk factors of CWD and further improve risk assessment and disease management ^[90]. The spread of CWD results in substantial economic losses to farming, gaming and tourism industries ^[11]. Once in the environment, prions retain their infectivity in soil for a prolonged period of time. This raises an ecological concern for potential cross-species transmission to other mammals located in the same geographic location ^[53] and public health concerns for the undetermined risk of human exposure to CWD through consumption of venison ^[60]. Therefore, it is vital to better understand the risk factors of CWD to improve risk assessment and potential disease management applications ^[70].

States surrounding Pennsylvania have dealt with CWD in wild deer and elk populations for decades. New York and West Virginia are the closest states to Pennsylvania where CWD has been detected ^[71]. CWD was first detected in Pennsylvania in 2012 at a captive deer facility and was soon after detected in free-ranging deer. During Pennsylvania's 2017-18 deer season, 51 deer harvested by hunters were tested positive for CWD. The disease stayed in the endemic areas of southern Blair, Bedford, and Fulton counties (Pennsylvania Game Commission CWD Report); all found in southcentral Pennsylvania. By developing a control program where officers work in these hotspots to remove infected animals and animals with a greater likelihood of carrying the disease is the best chance of controlling CWD on a larger scale.

5.4. Error Rates and Difficulties

While analyzing Case #2018-00040545, there was difficulty during the decalcification of the bone samples. Through trial and error, the proper amount of time needed for decalcification was determined. During initial attempts, the samples were decalcified over a total of 4-5 days. When samples were not yielding high quality DNA product, the samples

were re-extracted and decalcified for a total of 3 days. It was after these 3 days of decalcification where a high-quality DNA product was extracted.

To add, there is a new technique available to our laboratory that can be utilized in order to extract DNA from submitted bone samples in the future. The Forensic Department at Duquesne University has recently invested in a Barocycler, produced by Pressure Biosciences. Pressure Biosciences specializes in Pressure Cycler Technology (PCT) (PCT; Pressure BioSciences Inc., South Easton, MA, USA) and creates a variety of instruments to be used for Physical and Biological Sciences ^[69]. Pressure cycling has been shown to assist in the extraction of nucleic acids, proteins, lipids, and small molecules from cells and tissues ^[36]. During an investigation into the use of PCT to attempt to increase DNA yield in challenged samples, Marshall et al. (2013) observed that PCT reduced the effects of inhibition on downstream DNA analyses. Pressure generally has no effects on covalent bonds, and, therefore, natural compounds are typically not altered by high-pressure treatment at room temperature ^{[22] [66] [83]}.

Marshall et al. (2013) showed that PCT enhanced PCR efficiency for samples when compared to those samples not exposed to PCT. This study suggested that PCT had potential for forensic DNA analysis applications of challenged forensic DNA samples by reducing the effects of inhibitors known to be present in some bone samples ^[69].

5.5. Future Directions

There are new technologies on the horizon to pave the way for a new era in wildlife DNA forensics. For example, Next Generation Sequencing (NGS), has revolutionized the process of processing whole genomes by allowing these genomes to be sequenced more quickly at a lower cost ^[89]. Currently, the comparison of whole sequence data provides limited evidential value as closely related species will share much of their DNA ^[54]. The

process of NGS allows for the identification of repetitive DNA sequences and has led to significant reduction time for the identification of new highly informative markers, such as microsatellites ^[34] ^[57]. These new microsatellite loci can later be characterized for their polymorphic content, number of alleles, heterozygosity, linkage, and other relevant forensic parameters. NGS has recently been applied in a Research & Development capacity to investigate the content of some traditional medicines ^[18] that illegally utilize different species in creating these medicines. Once fully validated, this could become an important tool in investigating species content and even quantifying the content of samples. These new technologies will enable prompt discovery of molecular markers suitable for the identification of at the species, regional, and population levels ^[14]. The well-established techniques of population genetics will benefit future research in validating methods for DNA-based wildlife forensic identification ^[29]. It can be anticipated that DNA technologies will become an increasingly important tool in both national and international efforts to fight the illegal harvest and trade of wildlife ^[3].

The PGC has expressed a strong interest in being able to determine the likelihood that a deer came from a specific county, and so, through PCoA we were able to determine if this could be a possible service in the future. This service is of interest for the PGC due to the fact that there are many cases when an individual will shoot a deer in an area where they do not have a license to hunt and then transport the deer carcass back to the area where they are licensed to hunt. By law, a general hunting license is required to hunt in any season in the state of Pennsylvania. One antlered deer per license year can be taken with a general hunting license. Each hunter is limited to a maximum of three antlerless licenses in total, however, there is no limit on the number of licenses that can be obtained for WMUs 2B, 5C, and 5D. Hunters are only permitted to hunt in WMUs they purchased a license for (Pennsylvania Game Commission). Wildlife officers are able to tell, by rigor mortis, how long the deer has

been dead but would be able to more easily convict the perpetrator if forensic methods allowed them to pinpoint where the deer had geographically originated from.

Based on preliminary data in this thesis, a PCoA analysis has not provided the ability to determine if the clusters of samples on the plot originate from the same geographic location. For example, in Figure 1, samples Ovi0037 and Ovi0100 are found within the same cluster on the plot. However, when examining the WMU, county, and township of origin of these two samples, they are not the same. Ovi0037 was collected in WMU 2B in township 217, in Allegheny County while Ovi0100 was collected in WMU 2C in township 207, in Westmoreland County. Though this preliminary data suggests that PCoA is not a viable analysis to determine the geographic location of a sample, further analysis will be needed in order to confirm. If it is concluded that PCoA will not be used to provide this service to the Pennsylvania Game Commission, other methods will be considered and tested before ruling if our laboratory will be able to provide this service. For example, the program *Structure* is a software package used for multi-locus genotype data to investigate population structure. The programs' uses include inferring the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed. It can be applied to commonly used genetic markers, including microsatellites (*Structure* Software, Pritchard Lab, Stanford University, California). Additionally, a Population Assignment test in GenAlEx v6.5 may also be carried out as well to determine to geographic location of a sample.

Literature has shown a range of different loci used throughout the studies on white-tailed deer genetics. However, the loci used were common between studies, though not all studies utilized the same loci. To be more specific, there are 11 loci common between Florida, Michigan, Mississippi, Oklahoma, Pennsylvania, South Carolina, Texas, and West

Virginia ^[4] ^[26] ^[56] ^[76] ^[91] (Table 13). The wide range of common loci between these states allows us to believe that our panel will allow us to start to provide forensic services to states outside of Pennsylvania, such as: Ohio and West Virginia. In order to be able to widen our network, we will need to add additional loci to the current molecular panel to ensure a high degree of confidence when analyzing samples outside of the state of Pennsylvania.

Going forward, our laboratory will continue to establish a long-term wildlife forensics testing service in partnership with the Pennsylvania Game Commission. Doing so will provide resources needed for further evaluating the genetic structure of white-tailed deer across the state of Pennsylvania and will also provide forensic tools to neighboring state agencies.

Table 13: The observed number of alleles, observed Heterozygosity (H_O), and expected Heterozygosity (H_E) for eleven microsatellite loci common between white-tailed deer populations in Florida, Michigan, Mississippi, Oklahoma, Pennsylvania, South Carolina, Texas, and West Virginia.

		Florida, Mississippi, Oklahoma, Texas (DeYoung et al. 2003)	Michigan (Sorin 2004)	Oklahoma (Anderson et al. 2002)	Pennsylvania— Monroe County (Keeler et al. 2011)	Pennsylvania— Southwestern region	South Carolina (Comer et al. 2005)	West Virginia (Miller et al. 2010)
BM4208	Alleles	21	14	13	-	16	20	20
	H_O	0.80	0.85	0.95	-	0.98	0.86	0.92
	H_E	0.89	0.86	0.92	-	0.88	0.90	0.92
BM6506	Alleles	14	-	9	-	12	13	15
	H_O	0.71	-	0.68	-	0.53	0.82	0.86
	H_E	0.84	-	0.79	-	0.85	0.82	0.87
BM848	Alleles	20	-	10	13	-	-	-
	H_O	0.73	-	0.79	0.63	-	-	-
	H_E	0.81	-	0.83	0.80	-	-	-
Cervid1	Alleles	20	-	14	14	13	14	16
	H_O	0.83	--	0.83	0.74	0.75	0.84	0.83
	H_E	0.86		0.85	0.89	0.82	0.83	0.860
K	Alleles	10	-	3	7	-	7	-
	H_O	0.43	-	0.431	0.23	-	0.48	-
	H_E	0.45	-	0.452	0.67	-	0.46	-
N	Alleles	25	-	13	-	-	22	20
	H_O	0.72	-	0.82	-	-	0.79	0.853
	H_E	0.86	-	0.88	-	-	0.89	0.912
O	Alleles	9	-	4	4	-	6	8
	H_O	0.50	-	0.563	0.35	-	0.38	0.65
	H_E	0.59	-	0.51	0.56	-	0.43	0.64
P	Alleles	11	-	9	14	-	8	14
	H_O	0.76	-	0.46	0.77	-	0.79	0.85
	H_E	0.82	-	0.80	0.90	-	0.77	0.85
Q	Alleles	21	-	15	-	-	16	19
	H_O	0.83	-	0.80	-	-	0.87	0.819
	H_E	0.85	-	0.86	-	-	0.88	0.897
INRA011	Alleles	9	-	5	-	-	6	8
	H_O	0.63	-	0.68	-	-	0.59	0.55
	H_E	0.64	-	0.67	-	-	0.61	0.54
OarFCB193	Alleles	16	-	12	-	-	13	13
	H_O	0.88	-	0.85	-	-	0.61	0.909
	H_E	0.86	-	0.81	-	-	0.58	0.882

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Pennsylvania Game Commission 2009-2018 Deer Management Plan.

Pennsylvania Game Commission CWD Report.

Appendix

Appendix 1.1 Forms and Protocols

Case Submission Form

Janecka Genomics Laboratory

Department of Biological Sciences

Duquesne University

234 Mellon Hall, 913 Bluff Street, Pittsburgh PA, 15219

Contact Information:

Melanie Quain: [REDACTED] quainm@duq.edu

Dr. Jan E. Janecka: 412-396-5640 (Office/Lab) janeckaj@duq.edu

In order to process your casework, this form must be printed, filled out, and submitted with your evidence. Please call or email the laboratory prior to submitting samples. Samples can be delivered to the lab or we can pick them up at the Southwest Region PGC office. If you have any questions please do not hesitate to contact us.

NAME: _____

AGENCY: _____

DATE: _____

CASE NUMBER/REFERENCE CODE: _____

BRIEF DESCRIPTION OF INCIDENT: _____

Samples are processed based on their submission date. Expected turn-around time is 4-8 weeks. We will examine samples when they are received and provide you a more specific time estimate. If samples have unusually low quantity/quality DNA they may require additional processing and therefore supplemental charges may apply. In this situation we will notify you, explain the reasons for this, provide an estimate, and obtain your permission before performing any additional analysis that would increase the cost.

SELECT THE REQUESTED ANALYSIS (Cost per samples – we will provide you with a total before we process your samples)

- | | | | |
|-------|---|-------|---|
| _____ | A. Species Determination | \$100 | Determine the species of origin |
| _____ | B. Sex Determination | \$50 | Determine the sex of the animal |
| _____ | C. Individual Identification
(Bundled with Species and
Sex ID) | \$180 | Identify an individual based on a unique genetic
profile and match with other samples. |

_____	D. Minimum No. of Individuals	\$180	Determine the minimum number of individuals in a mixed sample, or several individual samples, based on genetic profiles
_____	E. Relatedness/Kinship	\$220	Estimate relatedness coefficient and test kinship

SUBMITTED SAMPLES:

	ID No.	Service Requested	Species	Species Known (Y or N)	Sex	Sex Known (Y or N)	Type of Sample
1)							
2)							
3)							
4)							
5)							
6)							
7)							
8)							
9)							
10)							

Indicate how you want final report: _____ **Email** _____ **Fax** _____ **Mail**

I hereby certify the information provided here in the submission form is accurate to the best of my knowledge. I understand I will be charged for the services requested based on the service fees and number of samples. I also understand the analysis and report will be provided by the Janecka Genomics Laboratory at Duquesne University in accordance with applicable standard procedures, terms, and conditions.

Name (Print): _____ Signature: _____
Date: _____

CONTACT INFORMATION	BILLING INFORMATION
Name:	Name:
Phone: Fax:	Phone: Fax:
Email:	Email:
Agency:	Agency:
Department:	Department:

Street:	Street:
City:	City:
State: Zip code:	State: Zip code:

Evidence Return: _____ (Please let us know where you would like samples returned.)
Appendix 1.2. Chain of Custody



Janecka Genomics Laboratory
Department of Biological Sciences, Duquesne University
234 Mellon Hall, 913 Bluff Street, Pittsburgh PA, 15219
412-396-5640 (Office/Lab), 570-730-5381, janeckaj@duq.edu, quainm@duq.edu

EVIDENCE CHAIN OF CUSTODY TRACKING FORM

Case Number: _____ Offense: _____

Submitting Officer: (Name/ID#) _____

Victim: _____

Suspect: _____

Date/Time Seized: _____ Location of Seizure: _____

Description of Evidence		
Item #	Quantity	Description of Item

I hereby acknowledge receipt to the listed evidence items which were taken into my custody on the listed date and received from the listed individual.

Chain of Custody				
Item #	Date/Time	Released by (Name/Signature)	Received by (Name/Signature)	Comments/Location

EVIDENCE CHAIN-OF-CUSTODY TRACKING FORM

(Continued)

Chain of Custody				
Item #	Date/Time	Released by (Name/Signature)	Received by (Name/Signature)	Comments/Location

Final Disposal Authority
<p>Authorization for Disposal</p> <p>Item(s) #: _____ on this document pertaining to (suspect): _____ is(are) no longer needed as evidence and is/are authorized for disposal by (check appropriate disposal method)</p> <p> <input type="checkbox"/> Return to Owner <input type="checkbox"/> Auction/Destroy/Divert </p> <p>Name & ID# of Authorizing Officer: _____ Signature: _____ Date: _____</p>
<p style="text-align: center;">Witness to Destruction of Evidence</p> <p>Item(s) #: _____ on this document were destroyed by Evidence Custodian _____ ID#: _____ in my presence on (date) _____.</p> <p>Name & ID# of Witness to destruction: _____ Signature: _____ Date: _____</p>
<p style="text-align: center;">Release to Lawful Owner</p> <p>Item(s) #: _____ on this document was/were released by Evidence Custodian _____ ID#: _____ to Name _____ Address: _____ City: _____ State: _____ Zip Code: _____ Telephone Number: (____) _____</p> <p>Under penalty of law, I certify that I am the lawful owner of the above item(s).</p> <p>Signature: _____ Date: _____</p> <p>Copy of Government-issued photo identification is attached. <input type="checkbox"/> Yes <input type="checkbox"/> No</p>
<p>This Evidence Chain-of-Custody form is to be retained as a permanent record by Duquesne University Wildlife Laboratory.</p>

Page 2 of 2 pages (See front)

Appendix 1.3. SOP – DNA Extraction

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory

SOP No: Ovi_18_001

**SOP Title: Purification of Total DNA in White-Tailed Deer
(*Odocoileus virginianus*) tissue the DNEasy® Blood & Tissue Kit**

SOP Number **Ovi_18_001**

SOP Title **Purification of Total DNA in White-Tailed Deer (*Odocoileus virginianus*)
tissue**

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

1. PURPOSE

This procedure is required in order to optimize the total amount of DNA recovered from all submitted samples. Those working on case samples must work in compliance with the standard operating procedure in order for sample analysis to be valid.

Successful recovery of DNA from biological evidence is the most important stage in any forensic investigation. Conservation geneticists have developed techniques to recover DNA from various sample types. This allows genetic information to be recovered from almost any biological matter.

2. RESPONSIBILITIES

Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

Individuals are to quantify the final Aliquots in a NanoDrop Lite Spectrometer to determine the DNA concentration and DNA purity.

Individuals are also expected to run an Agarose Gel after every set of extractions to verify that the DNA extracted is highly concentrated and pure.

3. SPECIFIC PROCEDURE

1. Cut up tissue into small pieces and place in a 1.5 ml Centrifuge tube
2. Add 180 µl Buffer ATL
3. Add 20 µl Proteinase K, mix by using the thermocycler at 56 °C until tissue is completely lysed (you may also just incubate and vortex occasionally)
 - a. Lysis time varies from 1-3 hours
4. Vortex for 15 seconds after incubation
5. Add 200 µl Buffer AL and mix thoroughly by vortexing
6. Add 200 µl of ethanol (96-100%) and mix thoroughly by vortexing
 - a. White precipitate may form an addition of Buffer AL and ethanol, this does not interfere with the results
7. Pipet mixture into DNEasy mini spin column placed in a 2 ml Collection tube
8. Centrifuge at $\geq 6000 \times g$ (8000rpm) for 1 minute
9. Discard flow through
10. Place DNEasy mini spin column in a new 2 ml Collection tube
11. Add 500 µl Buffer AW1 and centrifuge for 1 minute at $\geq 6000 \times g$ (8000 rpm)
12. Discard flow through
13. Place the DNEasy mini spin column in a new 2 ml collection tube
14. Add 500 µl Buffer AW2 and centrifuge for 3 minutes at 20,000 x g (14,000 rpm) to dry the DNEasy membrane
 - Important to dry the membrane of the DNEasy mini spin column
 - Following the centrifuge step, remove DNEasy mini spin column carefully so that the column does not come into contact with the flow through
15. Discard flow through
16. Place DNEasy mini spin column in a clean 1.5 ml or 2 ml centrifuge tube and pipet 200 µl Buffer AE directly onto the DNEasy membrane
17. Incubate at room temperature for 1 minute and then centrifuge at 1 minute at $\geq 6000 \times g$ (8000 rpm) to elute
 - a. For maximum yield in elution, repeat elution in Step 17

4. INTERNAL AND EXTERNAL REFERENCES

4.1 External References

http://diagnostics1.com/MANUAL/General_Qiagen.pdf

pages 28-30

Appendix 1.4. Purification of Total DNA from Compact Animal Bone

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory
SOP No: Ovi_18_002

**SOP Title: Purification of total DNA from compact
animal bone using the DNEasy® Blood and Tissue Kit**

SOP Number **Ovi_18_002**

SOP Title **Purification of total DNA from compact animal bone using the DNEasy®
Blood and Tissue Kit**

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

1. PURPOSE

This procedure is required in order to optimize the total amount of DNA recovered from all submitted samples. Those working on case samples must work in compliance with the standard operating procedure in order for sample analysis to be valid.

Successful recovery of DNA from biological evidence is the most important stage in any forensic investigation. Conservation geneticists have developed techniques to recover DNA from various sample types. This allows genetic information to be recovered from almost any biological matter.

2. RESPONSIBILITIES

Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

Individuals are to quantify the final Aliquots in a NanoDrop Lite Spectrometer to determine the DNA concentration and DNA purity.

Individuals are also expected to run an Agarose Gel after every set of extractions to verify that the DNA extracted is highly concentrated and pure.

3. SPECIFIC PROCEDURE

1. Completely remove bone marrow and soft tissues using sterilized drill and drill bit.
2. **If using ≤ 100 mg of powdered bone, follow step 2a; if using \geq follow step 2b**
 - 2a. Place up to 100 mg of powdered bone into a 2 mL microcentrifuge tube. Proceed immediately with **Step 6**.
 - 2b. Transfer 100 mg – 5g of the powdered bone into a sterile 50 mL polypropylene tube, and add 40 mL of 0.5 M EDTA, pH 7.5, to decalcify the sample. Agitate the tube on a rotator or rocking platform at 37° for 24 hours.
3. Centrifuge the sample at 2000 x g for 15 minutes. Discard the supernatant. Repeat the decalcification process several times.
 - **Generally, decalcification takes 3-5 days.**
4. Wash the pellet with 40 mL of sterile deionized water to remove ions that have accumulated during decalcification. Centrifuge the sample for 15 minutes at 2000 x g and discard the supernatant. Repeat this washing procedure 3 times.
5. Place up to 50 mg of the pellet into a 2 mL microcentrifuge tube.
6. Add 360 μ L Buffer ATL and 40 μ L proteinase K. Mix by vortexing, and incubate at 56°C until the pellet is completely lysed. Vortex occasionally during incubation to disperse the sample.
 - **Generally, lysis takes 3-5 days. Add 2 μ L of proteinase K every 24 hours.**
7. Vortex for 15 seconds. Add 400 μ L Buffer AL to the sample, and mix thoroughly by vortexing. Then add 400 μ L ethanol (96-100%), and mix again thoroughly by vortexing.
 - **It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogenous solution.**
 - **A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure.**
8. Pipet up to 650 μ L of the mixture from **step 7** (including any precipitate) into the DNeasy mini spin column placed in a 2 mL collection tube (provided). Centrifuge at ≥ 6000 x g (8000 rpm). Discard flow-through and collection tube.

9. Repeat **step 8** until all pf the sample has been loaded.
10. Place the DNeasy mini spin column in a new 2 mL collection tube (provided), add 500µL Buffer AW1, and centrifuge for 1 minute at $\geq 6000 \times g$ (8000 rpm). Discard flow-through and collection tube.
11. Place the DNeasy mini spin column in a new 2 mL collection tube (provided), add 500µL Buffer AW2, and centrifuge for 3 minutes at $20,000 \times g$ (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
 - **It is important to dry the membrane of the DNeasy mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.**
12. Place the DNeasy mini spin column in a clean 2 mL microcentrifuge tube (not provided), and pipet 200 µL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 minute, and then centrifuge for 1 minute at $\geq 6000 \times g$ (8000 rpm) to elute.

4. INTERNAL AND EXTERNAL REFERENCES

a. External References

<https://www.qiagen.com/mx/resources/resourcedetail?id=bd4e7285-9329-4158-9207-d37427a60940&lang=en>

pages 2-4

Appendix 1.5. Purification of Total DNA from Processed Meat and Animal Food Sample

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory
SOP No: Ovi_18_003
SOP Title: Purification of total DNA from animal food
sample using the DNEasy mericon® Food Kit

SOP Number **Ovi_18_003**
SOP Title **Purification of total DNA from animal food sample using the DNEasy**
 mericon® Food Kit

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	11/06/18
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

1. PURPOSE

This procedure is required in order to optimize the total amount of DNA recovered from all submitted samples. Those working on case samples must work in compliance with the standard operating procedure in order for sample analysis to be valid.

Successful recovery of DNA from biological evidence is the most important stage in any forensic investigation. Conservation geneticists have developed techniques to recover DNA from various sample types. This allows genetic information to be recovered from almost any biological matter.

2. RESPONSIBILITIES

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

Individuals are to quantify the final Aliquots in a NanoDrop Lite Spectrometer to determine the DNA concentration and DNA purity.

Individuals are also expected to run an Agarose Gel after every set of extractions to verify that the DNA extracted is highly concentrated and pure.

3. SPECIFIC PROCEDURE

1. Place 200 mg homogenized food sample in a 2 mL microcentrifuge tube; add 1 mL Food Lysis Buffer and 2.5 μ L Proteinase K solution. Vortex briefly to ensure complete distribution and moistening of the sample material.
 - To ensure that DNA yields are similar to those obtained using the standard protocol (2g), supernatants are pooled in step 5. Depending on the starting material, the supernatant from the 1 mL lysis solution will be less than 700 μ L. Be sure to prepare sufficient lysis tubes (in the range of 3-4 lysis tubes), so that supernatant aliquots from several lysis tubes can be pooled to draw the 700 μ L optimal for subsequent chloroform extraction.
2. Incubate in a thermomixer for 30 min at 60° C with constant shaking (1000 rpm). To enhance inhibitor precipitation, cool the sample to room temperature (15-25°C) on ice after incubation.
3. Centrifuge for 5 min at 2500 x g.
 - **The volume of supernatant strongly depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. A range of 200 μ L (homogenized foods) to 700 μ L (non-homogenized) can be expected after centrifugation.**
 - **Make sure not to carry over any precipitate from the bottom of the tube into the subsequent protocol steps.**
4. Pipet 500 μ L chloroform into a 2 mL microcentrifuge tube.
 - **Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.**
5. Carefully draw the maximum volume of clear supernatant from each lysis tube from **Step 3** without disturbing the inhibitor precipitate at the bottom of the tube. Combine the supernatant aliquots in one microcentrifuge tube and mix by pipetting up and down several times to ensure a homogenous solution.
6. Transfer 700 μ L of the clear supernatant pool to the microcentrifuge tube containing chloroform.
 - **The supernatant can be strongly colored. Certain foods may form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer only**

an aliquot of the clear middle phase. If the upper phase has formed a semi-solid film, pierce the film with the pipet and transfer only an aliquot of the clear middle phase.

7. Vortex the microcentrifuge tube from **step 6** vigorously for 15 seconds and centrifuge at 14,000 x g for 15 minutes.
 - **If the supernatant is not clear, centrifuge again for 5 minutes.**
8. Pipet 350 μ L Buffer PB into a fresh 2 mL microcentrifuge tube, add 350 μ L of the upper aqueous phase from **step 7** and mix thoroughly by vortexing.
9. Pipet the solution from **step 8** into the QIAquick spin column placed in a 2 mL collection tube. Centrifuge at 17,900 x g for 1 minute and discard the flow-through.
10. Reuse the collection tube from **step 9**. Add 500 μ L Buffer AW2 to the QIAquick spin column, centrifuge at 17,900 x g for 1 minute and discard flow-through. Reuse the collection tube and centrifuge again at 17,900 x g for 2 minute to dry the membrane.
 - **Residual ethanol from Buffer AW2 will not be completely removed unless the flow-through is discarded before the additional centrifugation.**
11. Transfer the QIAquick spin column to a 2 mL microcentrifuge tube (not supplied), and pipet 150 μ L Buffer EB directly onto the QIAquick membrane. Incubate for 1 minute at room temperature and then centrifuge at 17,900 x g for 1 minute to elute.

Appendix 1.6. Dilution of Primers to be used for PCR and genotyping of DNA samples

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory

SOP No: Ovi_18_004

**SOP Title: Dilution of primers to be used
for PCR and genotyping of DNA samples**

SOP Number Ovi_18_004

SOP Title Dilution of primers to be used for PCR and genotyping of DNA samples

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

1. PURPOSE

Primer tubes arrive in an un-hydrolyzed powder form that will need to be hydrolysed with TLE into a freezer stock solution and then diluted with 10 μM TRIS into a working stock solution.

2. RESPONSIBILITIES

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

3. SPECIFIC PROCEDURE

1. Take un-hydrolyzed primer tube and centrifuge at max Gs for 3 minutes to ensure that all powder product is at the bottom of the tube.
2. Open the tubes carefully and pipette the appropriate amount of TLE into primer tube. **CHANGE PIPETTE TIPS EACH TIME**
 - a. The amount of TLE you put in is dependent on the **nmols or Pmols that the un-hydrolyzed primer** came as (you can find this on the primer information sheet) and **the concentration you want your working stock to be**.
 - i. If primers come in nmol concentration, and you want 200 μM working stock, take the nmols of each primer and multiply by 5 to get the μL of TLE to be added.
 1. FOR EXAMPLE: primer 1F(17.9 nmols) $\rightarrow 17.9 \text{ nmols} \times 5 = 89.5 \mu\text{L}$ of TLE to add to hydrate primers to 200 μM working stock.
3. Once you add the TLE, label the freezer stock tubes that your primers came in with the concentration on the side and the lid of the tube (ie. “200 μM ”) and “Freezer Stock” on the side.
4. Vortex/shake freezer stock tubes for 10 minutes at a speed of 800-1,000.
5. During this 10-minute period, label the **sides** of your working stock primer tubes with the primer name, date, and concentration and the **lids** with the concentration (you can also put the name on here if you can fit it).
 - a. FOR EXAMPLE: “Primer1F 01-29-18 20 μM ” on side ... “20 μM ” on top.
6. When your freezer stock tubes are done on the vortexer, spin them down via a pulse spin in the centrifuge.
7. You will need to figure out how much TRIS to add to make your working stock primers 20 μM . This can be done with the following two-part equation:
 - a. (The concentration you want/ Divided by the concentration you have) **The total volume you want = the amount of freezer stock to add in μL**
 - b. The total volume you want – the amount freezer stock to add in μL = the amount of TRIS to add in μL .
 - i. FOR EXAMPLE: $(20 \mu\text{M} / 200 \mu\text{M}) \times 100 = 10 \mu\text{L}$ of 200 μM freezer stock;
 $100 - 10 \mu\text{L}$ of 200 μM freezer stock = 90 μL of TRIS to add to dilute to 20 μM .
8. Line up your freezer stock tubes in a rack to pair up with your working stock tubes to avoid confusion when pipetting from freezer stock tubes to working stock tubes.
 - a. FOR EXAMPLE: place “Primer1F Freezer Stock” tube behind “Primer1F Working Stock” tube.
9. Pipette the amount of freezer stock primer you calculated from your freezer stock tube into the corresponding labelled working stock tube. **Change pipette tips after each tube**
10. Pipette the amount of TRIS you calculated. **Change the pipette tips after each tube**

11. Wrap foil around the bottom of your forward primers. The forward primers have a fluorescent tag that is light sensitive. Wrapping in foil helps to prevent the degradation of the fluorescent tag.
12. Put your primers in a box and label the box appropriately with the project and name (ie. "Freezer and Working stock Primers for Cervid Analysis Melanie Quain". Place in -20 freezer for future use.

Appendix 1.7. Microsatellite PCR and Fragment Analysis of PCR Amplicons

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory

SOP No: Ovi_18_005

SOP Title: Microsatellite PCR and Fragment Analysis of PCR Amplicons

SOP Number **Ovi_18_005**

SOP Title **Preparation and dilution of PCR samples to genotype**

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

1. PURPOSE

This procedure is required in order to genotype samples efficiently.

2. RESPONSIBILITIES

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

3. SPECIFIC PROCEDURE

Preparing PCR samples

1. Vortex all of your samples to make the samples homogenous.
2. Centrifuge to get liquid to the bottom of the tube.
3. Set up your PCR excel sheet and print it out.
4. Make your Master Mix. **The “Recipe” is listed on PCR excel sheet**
5. Add 1.5 μL of DNA into each well. Correspond each sample with the table on your excel sheet.
6. Add 8.5 μL of your Master Mix to each well.
7. Cover the plate with film.
8. Spin down the plate.
9. Place in the thermocycler. **Adjust on the screen as needed**
10. Run your PCR gel **2 μL of loading dye and 4 μL of DNA**

Dilution of PCR amplicons

1. Add 120 μL of MiliQ H2O or ddH2O into each well of your 96 well plate.
2. Add 1.5 μL of product (PCR sample and MilliQ H2O mixture) into skirted plate.
3. Add 8 μL of Liz/formamide mix

970 μL formamide

30 μL Liz

Vortex for 2 min ... then spin down in centrifuge

Appendix 1.8. Sex ID PCR

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory
SOP No: Ovi_18_006
SOP Title: Sex ID PCR

SOP Number **Ovi_18_006**
SOP Title **Sex ID PCR**

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

4. PURPOSE

This procedure is required in order to sex ID samples efficiently.

5. RESPONSIBILITIES

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

6. SPECIFIC PROCEDURE

Preparing Sex ID PCR samples

11. Vortex all of your samples to make the samples homogenous.
12. Centrifuge to get liquid to the bottom of the tube.
13. Set up your PCR excel sheet and print it out.
14. Make your Master Mix. **The “Recipe” is listed on PCR excel sheet**
15. Add 1.5 μL of DNA into each well. Correspond each sample with the table on your excel sheet.
16. Add 8.5 μL of your Master Mix to each well.
17. Cover the plate with film.
18. Spin down the plate.
19. Place in the thermocycler. **Adjust on the screen as needed**
20. Run your PCR gel **2 μL of loading dye and 4 μL of DNA**

Appendix 1.9. Species ID PCR; Ultra-Clean PCR Clean-Up; Species Sequencing; EtOH/EDTA/Sodium Acetate Precipitation; and, Species Identity

**STANDARD
OPERATING
PROCEDURE**

Duquesne University Janecka Genomics Laboratory

SOP No: Ovi_18_007

SOP Title: Species ID PCR; Ultra-Clean PCR Clean-Up; Species Sequencing; EtOH/EDTA/Sodium Acetate Precipitation; and, Species Identity

SOP Number **Ovi_18_006**
SOP Title **Sex ID PCR**

	NAME	TITLE	SIGNATURE	DATE
Author	Melanie Quain	M.S. Student		
Reviewer				
Authoriser				

Effective Date:	10/01/2018
Review Date:	

READ BY			
NAME	TITLE	SIGNATURE	DATE

7. PURPOSE

This procedure is required in order to identify the species of the samples efficiently.

8. RESPONSIBILITIES

Those following this protocol are expected to follow the steps below while avoiding contamination of samples.

9. SPECIFIC PROCEDURE

Preparing Species ID PCR samples

21. Vortex all of your samples to make the samples homogenous.
22. Centrifuge to get liquid to the bottom of the tube.
23. Set up your PCR excel sheet and print it out.
24. Make your Master Mix **“Recipe” is listed on PCR excel sheet**
25. Add 1.5 μL of DNA into each well. Correspond each sample with the table on your excel sheet.
26. Add 8.5 μL of your Master Mix to each well.
27. Cover the plate with film.
28. Spin down the plate.
29. Place in the thermocycler. **Adjust on the screen as needed**
30. Run your PCR gel **2 μL of loading dye and 4 μL of DNA**

Ultra-Clean PCR Clean-Up

1. Shake to mix the SpinBind before use. Add 5 volumes of the SpinBind to your PCR reaction.
2. Mix by pipetting.
3. Transfer PCR/SpinBind mixture to a Spin Filter unit, while avoiding the transfer of oil.
4. Centrifuge for 10-30 seconds at 13,000 rpm.
5. Remove the Spin Filter basket and discard the flow-through from the tube by decanting.
6. Place the Spin Filter basket back into the same tube.
7. Add 300 μL of SpinClean buffer into the Spin Filter.
8. Centrifuge for 10-30 seconds at 13,000 rpm.
9. Remove the Spin Filter basket and discard the flow-through by decanting.
10. Place the Spin Filter basket back into the same tube.
11. Transfer the Spin Filter into a clean 2.0mL collection tube.
12. Add 50 μL of H_2O , to elute, onto the center of the Spin Filter membrane.
13. Centrifuge for 30-60 seconds at 13,000 rpm.
14. Discard the Spin Filter basket.
15. The purified DNA is now in the 2.0mL collection tube.

Species Sequencing

1. Set up PCR excel sheet and print it out.

2. Make your Master Mix. **The “Recipe” is listed on the PCR excel sheet.**
3. Add 1.5 μL of DNA into each well. Correspond each sample with the table on your excel sheet.
4. Add 8.5 μL of your Master Mix to each well.
5. Cover the plate with film.
6. Spin down the plate.
7. Place in the thermocycler. **Adjust on the screen as needed.**
8. Remove excess dNPs with EtOH/EDTA/Sodium Acetate precipitation.

EtOH/EDTA/ Sodium Acetate Precipitation

1. Prepare a 2.0mL microcentrifuge tube containing: 2 μL of 3M sodium acetate, pH 5.2 and 2 μL of 125mM EDTA, pH 8.0.
2. Pipette contents of each sequencing reaction into the tube of sodium acetate/EDTA.
3. Vortex briefly.
4. Add 50 μL of 100% EtOH into each tube.
5. Vortex and spin briefly.
6. Incubate the tube(s) at room temperature for 15 minutes.
7. Centrifuge for 20 minutes at maximum speed.
8. Carefully aspirate the solution from the pellet with a pipette tip and discard.
9. Spin down the tube(s) again to remove any residual solution, if required.
10. Rinse the pellet by adding 250 μL of 70% EtOH.
11. Vortex briefly.
12. Spin the tube(s) for 5 minutes at maximum speed.
13. Carefully aspirate any liquid away from the pellet.
14. Spin down the tube(s) again to remove any remaining solution, if required.
15. Dry the pellet(s) completely by air drying on the bench.
16. Add 10 μL of Formamide.
17. Vortex for 2 minutes and then spin down in centrifuge.

Species Identity

1. Run sample(s) from EtOH/EDTA/Sodium Acetate Precipitation protocol and run on ABI 3130 sequencer using sequencing protocol.
2. Import raw data to MEGA7.
3. Blast sequences against the NCBI GenBank database.
4. The sequence identity and E-value of the best hit is used to determine what species the sample is from.

Appendix 2. White-tailed deer sample catalog

Catalog #	Date Collected/ Killed	Sex	Est. Age	County #	Township #	WMU
Ovi0001	-	F	-	02	466	2B
Ovi0002	-	F	-	02	113	2B
Ovi0003	3/17/2009	F	-	04	411	2B
Ovi0004	-	F	-	02	217	2B
Ovi0005	-	F	-	02	108	2B
Ovi0006	-	F	-	02	216	2B
Ovi0007	3/10/2009	F	Y-5 years	Allegheny	Forward	2B
Ovi0008	-	F	-	02	412	2B
Ovi0009	-	F	-	02	432	2B
Ovi0010	3/31/2009	M	2 years	Allegheny	White Oak	2B
Ovi0011	-	F	-	02	108	2B
Ovi0012	-	F	-	02	216	2B
Ovi0013	3/17/2009	F	-	04	202	2B
Ovi0014	5/18/2009	M	-	04	203	1A
Ovi0015	4/8/2009	F	-	04	211	1A
Ovi0016	-	F	-	04	212	1A
Ovi0017	5/4/2009	F	3 years	Allegheny	Monroeville	2B
Ovi0018	-	F	-	02	216	2B
Ovi0019	-	M	-	02	108	2B
Ovi0020	3/20/2009	M	-	02	481	2B
Ovi0021	-	M	-	02	481	2B
Ovi0022	04/14/2009	M	1 year	Allegheny	North Fayette	2B
Ovi0023	03/17/2009	F	-	04	217	1A
Ovi0024	04/13/2009	F	1 year	Allegheny	Moon	2B
Ovi0025	03/01/2009	M	1 year	Allegheny	Overbrook	2B
Ovi0026	02/03/2009	F	2 years	Allegheny	Elizabeth	2B
Ovi0027	03/20/2009	M	1 year	Washington	Peters	2B

Catalog #	Date Collected/ Killed	Sex	Est. Age	County #	Township #	WMU
Ovi0028	-	F	-	02	466	2B
Ovi0029	-	F	-	04	211	2B
Ovi0030	-	F	-	02	108	2B
Ovi0031	-	M	-	02	113	2B
Ovi0032	-	F	-	02	212	2B
Ovi0033	-	F	-	02	108	2B
Ovi0034	-	F	-	02	216	2B
Ovi0035	-	F	-	02	216	2B
Ovi0036	-	M	-	02	481	2B
Ovi0037	-	F	-	02	217	2B
Ovi0038	-	F	-	02	108	2B
Ovi0039	-	M	-	02	481	2B
Ovi0040	02/24/2009	F	1 year	Allegheny	Upper St. Clair	2B
Ovi0041	-	F	-	02	466	2B
Ovi0042	-	M	-	02	113	2B
Ovi0043	05/16/2009	F	-	04	103	2B
Ovi0044	-	M	-	02	215	2B
Ovi0045	02/24/2009	F	1 year	Allegheny	Moon	2B
Ovi0046	02/25/2009	F	2 years	Allegheny	Findlay	2B
Ovi0047	07/22/2018	F	5 years	02	431	2B
Ovi0048	07/11/2018	F	2 years	02	105	2B
Ovi0049	07/18/2018	M	2 years	02	431	2B
Ovi0050	-	F	-	02	220	2B
Ovi0051	02/24/2009	F	1 year	Allegheny	Upper St. Clair	2B
Ovi0052	-	M	-	02	216	2B
Ovi0053	-	F	-	02	108	2B
Ovi0054	-	F	-	02	108	2B

Catalog #	Date Collected/ Killed	Sex	Est. Age	County #	Township #	WMU
Ovi0055	-	F	-	02	220	2B
Ovi0056	-	F	-	02	432	2B
Ovi0057	-	F	-	02	212	2B
Ovi0058	-	F	-	02	116	2B
Ovi0059	-	-	-	02	216	2B
Ovi0060	-	M	-	02	212	2B
Ovi0061	-	M	-	02	481	2B
Ovi0062	-	M	-	02	216	2B
Ovi0063	-	M	-	02	216	2B
Ovi0064	-	F	-	02	220	2B
Ovi0065	-	F	-	02	212	2B
Ovi0066	03/15/2009	F	-	Allegheny	West Mifflin	2B
Ovi0067	-	F	-	02	108	2B
Ovi0068	03/01/2009	F	1 year	Allegheny	Moon	2B
Ovi0069	03/01/2009	F	1 year	Allegheny	Moon	2B
Ovi0070	04/02/2009	F	1 year	Allegheny	Emsworth	2B
Ovi0071	04/03/2009	F	1 year	Allegheny	Edgeworth	2B
Ovi0072	04/03/2009	F	2 years	Allegheny	Leet	2B
Ovi0073	03/10/2009	F	1 year	Allegheny	S. Fayette	2B
Ovi0074	-	M	-	Allegheny	Leet	2B
Ovi0075	08/27/2018	M	2 years	03	227	2D
Ovi0076	07/02/2018	M	Juvenile	26	208	2C
Ovi0077	08/29/2018	M	Adult	11	-	2C
Ovi0078	08/24/2018	M	Adult	56	209	2C
Ovi0079	08/10/2018	-	Juvenile	56	225	2C
Ovi0080	08/02/2018	F	Adult	26	214	2A
Ovi0081	08/09/2018	F	Juvenile	65	212	2A

Catalog #	Date Collected/ Killed	Sex	Est. Age	County #	Township #	WMU
Ovi0082	08/04/2018	F	Fawn	65	203	2C
Ovi0083	08/09/2018	F	Adult	02	213	2B
Ovi0084	07/03/2018	-	Juvenile	56	213	2C
Ovi0085	08/08/2018	F	Adult	04	209	2A
Ovi0086	08/25/2018	M	2 years	03	202	2D
Ovi0087	07/10/2018	F	3 years	26	210	2A
Ovi0088	07/13/2018	M	Juvenile	04	405	1A
Ovi0089	08/23/2018	-	Juvenile	02	217	2B
Ovi0090	08/23/2018	F	3 years	02	216	2B
Ovi0091	07/13/2018	M	Adult	04	405	1A
Ovi0092	08/05/2018	F	2 years	03	219	2D
Ovi0093	07/25/2018	-	Fawn	02	301	2B
Ovi0094	07/10/2018	M	2 years	65	216	2A
Ovi0095	08/07/2018	F	3 years	02	108	2B
Ovi0096	08/07/2018	-	Fawn	02	116	2B
Ovi0097	07/03/2018	F	Adult	32	227	2D
Ovi0098	07/27/2018	M	Adult	65	207	2C
Ovi0099	07/20/2018	M	Adult	65	217	2C
Ovi0100	07/04/2018	F	Adult	65	207	2C
Ovi0101	06/29/2018	M	Juvenile	65	204	2C
Ovi0102	07/21/2018	M	Adult	65	204	2C
Ovi0103	07/16/2018	-	Juvenile	32	222	2C
Ovi0104	07/09/2018	M	Adult	56	-	2C
Ovi0105	07/02/2018	-	Juvenile	56	218	2C
Ovi0106	07/02/2018	F	Adult	56	218	2C
Ovi0107	08/04/2018	F	Adult	56	210	2C
Ovi0108	08/14/2018	F	Fawn	65	209	2C
Ovi0109	08/14/2018	F	Fawn	65	408	2B

Appendix 3. Genotypes of Reference Samples for Genetic Variation Analysis

Sample	BM6506	RT7	BM4208	BM1225	Cervid1F	RT24	Bm4107
Ovi0001	191/197	215/219	143/161	229/229	192/196	219/223	145/157
Ovi0002	191/191	221/225	169/175	231/239	176/180	213/219	147/157
Ovi0003	171/171	223/225	169/171	-	172/192	213/223	143/163
Ovi0004	193/199	225/229	143/143	235/237	174/174	219/231	139/143
Ovi0005	193/203	217/219	145/159	235/235	-	-	-
Ovi0006	203/203	209/227	143/149	229/237	174/192	213/223	159/167
Ovi0007	191/193	207/219	143/143	231/231	182/188	211/217	143/147
Ovi0008	185/191	221/227	165/171	-	180/182	213/213	145/147
Ovi0009	189/189	227/231	169/169	-	192/192	213/213	139/157
Ovi0010	191/199	209/217	143/161	235/235	180/192	213/217	157/157
Ovi0011	193/201	209/217	165/165	229/231	172/180	213/219	139/143
Ovi0012	185/203	225/231	143/169	229/239	172/172	213/223	143/155
Ovi0013	193/193	219/225	171/171	231/233	-	-	-
Ovi0014	185/185	209/227	161/169	233/233	174/190	213/213	139/147
Ovi0015	191/203	205/231	171/175	-	182/192	213/213	143/143
Ovi0016	193/193	217/219	173/179	231/237	178/186	219/225	143/143
Ovi0017	199/199	207/227	163/177	235/237	180/196	207/207	143/155
Ovi0018	191/191	215/223	171/171	229/235	172/180	213/213	143/147
Ovi0019	179/195	-	207/207	231/231	180/182	213/215	139/155
Ovi0020	-	-	-	-	168/174	207/213	143/165
Ovi0022	199/199	223/225	143/163	235/235	174/186	207/217	143/167
Ovi0023	185/193	221/227	175/177	231/237	174/192	211/213	143/143
Ovi0024	-	209/231	161/175	231/237	190/190	215/225	155/157
Ovi0025	185/195	219/225	169/181	-	174/176	213/215	141/143
Ovi0026	193/193	227/227	169/179	229/229	168/172	215/215	155/155
Ovi0027	-	-	-	-	174/176	213/215	141/143
Ovi0028	199/199	217/223	169/181	229/231	182/182	219/223	157/165
Ovi0029	189/193	209/217	177/177	231/237	172/182	215/233	143/155

Sample	BM6506	RT7	BM4208	BM1225	Cervid1F	RT24	BM4107
Ovi0030	199/199	219/223	145/179	229/239	172/192	219/227	153/157
Ovi0031	171/171	223/225	143/143	239/239	174/190	215/225	139/147
Ovi0032	193/193	227/229	163/167	225/225	-	-	-
Ovi0033	185/199	225/227	145/145	-	182/182	215/219	147/159
Ovi0034	189/199	217/223	175/175	-	162/182	213/213	143/155
Ovi0035	193/199	225/229	175/175	229/229	168/174	213/227	143/143
Ovi0037	171/199	203/225	179/179	-	-	-	-
Ovi0038	189/189	217/227	171/171	-	174/182	213/213	155/155
Ovi0039	193/199	217/225	145/161	-	174/192	213/227	147/147
Ovi0040	191/195	223/225	173/173	235/237	174/176	-	-
Ovi0041	199/209	225/231	143/143	229/235	172/172	213/213	143/163
Ovi0042	185/199	209/227	177/177	231/237	188/190	215/215	157/157
Ovi0043	199/201	215/219	169/169	237/237	174/178	207/223	143/157
Ovi0044	191/193	219/225	145/177	229/237	178/182	-	-
Ovi0045	199/199	-	-	-	174/174	219/223	143/157
Ovi0046	193/297	219/225	171/175	225/225	174/180	213/217	155/157
Ovi0047	187/191	215/221	167/169	231/231	166/172	205/211	147/153
Ovi0048	185/193	221/223	161/161	229/231	170/170	209/215	141/143
Ovi0049	191/199	225/233	163/171	225/235	178/178	211/223	-
Ovi0075	193/203	223/225	143/143	231/237	170/180	213/213	141/155
Ovi0076	-	-	-	-	180/180	211/217	145/153
Ovi0077	193/193	227/227	147/147	237/237	178/190	211/215	141/155
Ovi0078	197/199	225/225	-	229/237	178/190	205/211	153/153
Ovi0079	189/189	225/225	163/163	231/239	176/176	211/219	145/145
Ovi0080	193/193	223/229	143/143	231/237	160/174	-	-
Ovi0081	191/191	225/231	165/167	229/239	166/178	209/213	-
Ovi0082	201/203	207/215	169/169	231/231	-	209/209	-
Ovi0083	197/197	225/225	147/177	229/237	178/184	207/209	141/155
Ovii0084	189/189	209/219	157/167	231/237	170/170	201/209	141/145

Sample	BM6506	RT7	BM4208	BM1225	Cervid1F	RT24	BM4107
Ovi0085	199/199	219/299	169/177	231/237	178/178	211/217	141/153
Ovi0086	195/197	209/225	147/147	235/235	172/180	211/221	141/141
Ovi0087	193/193	219/231	-	-	172/182	211/213	141/163
Ovi0088	193/199	219/231	167/171	235/237	178/180	215/215	141/159
Ovi0089	193/201	225/225	147/147	231/233	178/180	205/215	143/155
Ovi0090	-	215/215	147/177	229/231	178/180	217/219	141/159
Ovi0091	187/197	221/227	147/147	227/235	176/180	211/221	141/153
Ovi0092	191/191	215/221	143/161	231/239	172/182	209/217	139/145
Ovi0093	185/191	219/223	177/179	235/235	172/172	217/221	139/139
Ovi0094	-	215/225	-	237/237	158/190	209/221	143/163
Ovi0095	189/189	217/225	171/175	229/235	160/180	211/221	141/145
Ovi0096	189/193	219/225	143/171	229/231	170/178	211/211	153/155
Ovi0097	-	-	-	-	172/180	211/211	141/147
Ovi0098	199/199	217/221	165/167	229/235	166/166	209/209	153/155
Ovi0099	197/203	225/229	147/163	229/237	160/172	205/219	143/155
Ovi0100	187/195	209/217	143/157	-	160/166	-	-
Ovi0101	185/185	227/227	169/169	231/237	178/182	207/217	143/155
Ovi0102	201/203	225/225	147/157	229/231	176/180	211/221	141/145
Ovi0103	197/199	225/225	167/167	-	178/180	211/215	141/145
Ovi0104	193/203	225/231	165/167	229/237	178/184	205/211	143/155
Ovi0105	197/197	219/229	165/167	229/231	-	-	-
Ovi0106	201/203	215/217	147/167	229/237	172/180	205/215	141/141
Ovi0107	193/193	219/231	169/177	231/231	172/180	211/221	141/141
Ovi0108	197/199	225/231	147/157	-	172/180	207/209	153/153
Ovi0109	201/203	223/229	161/161	-	158/172	221/221	141/163