Grand Valley State University
ScholarWorks@GVSU

Masters Theses | Graduate Research and Creative Practice

5-11-2022

Genetic Structure of Urban White-Tailed Deer in West Michigan

Jacob David Brand
Grand Valley State University

Follow this and additional works at: https://scholarworks.gvsu.edu/theses

Part of the Genetics Commons, Natural Resources and Conservation Commons, and the Natural Resources Management and Policy Commons

ScholarWorks Citation
Brand, Jacob David, "Genetic Structure of Urban White-Tailed Deer in West Michigan" (2022). *Masters Theses*. 1045.
https://scholarworks.gvsu.edu/theses/1045

This Thesis is brought to you for free and open access by the Graduate Research and Creative Practice at ScholarWorks@GVSU. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks@GVSU. For more information, please contact scholarworks@gvsu.edu.

Genetic Structure of Urban White-Tailed Deer in West Michigan

Jacob Brand

A Thesis Submitted to the Graduate Faculty of

GRAND VALLEY STATE UNIVERSITY

In

Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Biology

Biology Department

April 2022

Thesis Approval Form

The signatories of the committee members below indicate that they have read and approved the thesis of Jacob David Brand in partial fulfillment of the requirements for the degree of Master of Science.

05/05/2022

Dr. Alexandra Locher, Thesis committee chair Date

05/05/2022

Dr. Georgette Sass, Committee member Date

5/4/2022

Dr. Charlyn Partridge, Committee member Date

05/05/2022

Dr. Jennifer Moore, Committee member Date

Accepted and approved on behalf of the College of Liberal Arts and Sciences

Dean of the College

5/11/2022

Date

Accepted and approved on behalf of the Graduate Faculty

Associate Vice-Provost for the Graduate School

5/11/2022

Date

DEDICATION

I would like to dedicate this to my family. You have always supported me through my academic endeavors. I would not have been able to accomplish any of this without you. I would also like to dedicate this to my dogs Moose and Koda who kept me company and slept on my feet throughout the entire process.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank my committee members Dr. Alexandra Locher, Dr. Georgette Sass, Dr. Charlyn Partridge, and Dr. Jennifer Moore for their dedication to this project and support along the way. I would like to give a special thank you to Dr. Eric McCluskey for his coding expertise throughout this project and to Jordan Veldboom for his help in the genetics lab.

ABSTRACT

In urban areas, green spaces are used by humans and wildlife. The proximity between them can lead to both positive and negative interactions, which can make managing urban wildlife difficult. Managers are challenged due to conflicts between wildlife population sizes that can be naturally supported versus those that are socially tolerable. White-tailed deer (*Odocoileus virginianus*) thrive in urban environments because their habitat requirements are met within green spaces and backyard vegetation. Matrilineal groups of urban white-tailed deer live within the same areas, at times forming high densities that can lead to the spread of diseases or environmental pests, including hemlock wooly adelgid and ticks. Understanding the relatedness between those matrilineal groups may assist managers in understanding their movement patterns and help identify management strategies. Technological advancements in genetics have allowed researchers to investigate wildlife population structure at a molecular level and use that information in population management. The objective of this study was to understand the genetic structure of an urban deer population to determine baseline population data on which to make management decisions. We investigated deer population genetics from fecal samples collected throughout Grand Haven, Michigan, and the surrounding residential areas. Using microsatellites in a mark-recapture study, we assessed genetic clustering, sex ratios and calculated a population estimate. Results revealed 5 genetic clusters, a 24:1 female to male sex ratio and a population density of approximately 15 deer/ km^2 (range = 7.9 – 21.9 deer/km^2). Mantel tests showed a positive correlation between distance and genetic diversity within our study area. Results may be used in future landscape genetics research to investigate potential features inhibiting or facilitating gene flow within urban areas.

TABLE OF CONTENTS

TITLE PAGE 1
THESIS APROVAL FORM 2
DEDICATION 3
ACKNOWLEDGEMENT 4
ABSTRACT 5

LIST OF TABLES 7

LIST OF FIGURES 8

ABBREVIATIONS 9

CHAPTER 1. INTRODUCTION 10
- Purpose 13
- Scope 13
- Assumptions 14
- Objectives 14
- Significance 15

CHAPTER 2. GENETIC STRUCTURE OF URBAN WHITE-TAILED DEER IN WEST MICHIGAN 16
- ABSTRACT 16
- KEY WORDS 17
- STUDY SITE 19
- METHODS 21
  - Sample Collection and Processing 21
  - Sex Determination 22
  - Microsatellite Panel 23
  - Population Estimates and Structure 24
- RESULTS 26
- DISCUSSION 27
- MANAGEMENT IMPLICATIONS 34
- ACKNOLWLEDGEMENTS 35
- LITERATURE CITED 35

- Figure Captions 42

CHAPTER 3. EXTENDED REVIEW OF LITERURE AND METHODOLOGY 52
- Extended Review of Literature 52
- Extended Review of Methodology 60

APPENDICES 67

BIBLIOGRAPHY 76

LIST OF TABLES

Chapter 2.

Table 1. Microsatellite panels for white-tailed deer used for this research based on previous research conducted by Crawford et al. (2018) and Anderson et al. (2002). Each fluorescently-labeled microsatellite was placed into groups based on the fluorescent tags and amplicon size range. The annealing temperatures for our polymerase chain reactions are listed.

Table 2. Landcover categories from C-CAP 2016 data within a 65 km^2 area for the cities of Grand Haven, Ferrysburg, and Spring Lake, Michigan. Each landcover has the percent area and the total area in hectares reported along with a description of the features within the cover type.

Table 3. Number of white-tailed deer fecal samples collected by season (2020-2021) within predominant land cover types in a 65 km^2 area including the cities of Grand Haven, Ferrysburg, and Spring Lake, Michigan.

Chapter 3.

Table 1. The ten microsatellites were split into two groups based on the number of amplicon size in base pairs and the fluorescent tag for the forward primer. The microsatellites were pooled into two 96-well plates corresponding with their group for fragment analysis.

LIST OF FIGURES

Chapter 2.

Figure 1. Study area boundary (65 km^2) encompassing the city of Grand Haven, Michigan (focal city), with Ferrysburg and Spring Lake to the north.

Figure 2. Locations of white-tailed deer fecal pellets collected during 2020-2021 for a population-genetics study. Pellet collection locations were stratified relative to US Highway 31 (east-west division) and The Grand River (north-south division).

Figure 3. Land cover composition within 65 km^2 study area in west Michigan. Land cover categories were based on C-CAP 2016 data.

Figure 4. BIC values for the number of potential subpopulations with the male and female subsets of white-tailed deer near Grand Haven, Michigan (2021).

Figure 5. An assignment plot based on the level of K determined by running DAPC for male and female white-tailed deer near Grand Haven, Michigan (2021).

Figure 6. BIC values for the number of potential subpopulations of white-tailed deer near Grand Haven, Michigan (2021) based on only females from the 148-sample subset.

Figure 7. An assignment plot for female white-tailed deer in urban areas near Grand Haven, Michigan (2021) based on the number of subpopulations after running the DAPC.

ABBREVIATIONS

BIC- Bayesian Information Criterion

DAPC- Discriminate Analysis of Principal Components

DNA- Deoxyribonucleic acid

eDNA- Environmental Deoxyribonucleic acid

GPS- Global Positioning System

PCR- Polymerase Chain Reactions

SB Sodium Borate

SRY- Sex-determining Region of the Y-Chromosome

CHAPTER 1. INTRODUCTION

Cities in the United States are a patchwork of developed areas interspersed with patches of wildlife habitat. These patches of habitat can include public parks, back yards, and woodlots found throughout the boundaries of the cities. In 2008 over half of the world's human population lived in urban environments (Goddard et al. 2009). This number continues to grow as the global population continues to increase.

Over the last 80 years the human population in the United States has increased from 150 million to 332 million people. With the population boom in the US there has been a need for increased living spaces. This need has caused an expansion of urban and suburban residences into rural locations. As of 2019, 82% of the population in the US lives in urban settings. Michigan is host to 10 of the 332 million residents. The population of Michigan has grown by 2 percent in the last decade which is slower than the Midwest average of 3.1% increase. Roughly 74% of 10 million Michigan residents live within these urban areas.

The influx of citizens to these urban areas have caused the cities to expand and overtake the surrounding rural areas. In the 2000 fiscal budget Clinton proposed increased spending on conservation and green spaces to help preserve and conserve natural areas within and surrounding our cities. As the urban footprint expands outside of the cities more land is converted from forested to agricultural fields. The conversion of land reduces the amount of biodiversity that animals rely on. Green spaces within cities create an island that provides animals the life requisites needed for survival.

Many wildlife species, can survive in suburban, exurban, and rural landscapes, but their selection of habitat requirements may differ. Suburban landscapes generally mean the cities and suburbs surrounding them while exurban is the agricultural and less densely populated land

surrounding the suburban sections of cities (Storm et al. 2007). The main difference between habitat patches for exurban and urban locations is that in exurban areas human development is interspersed into wildlife habitat whereas cities contain smaller wildlife habitat patches within the city limits (Odell and Knight 2001). For example, white-tailed deer (*Odocoileus virginianus*) occupy a variety of environments by seeking habitat requisites from rural forests and woodlots, to urban parks, greenspaces, and residential gardens as long as those areas provide adequate food and cover (Kilpatrick and Spohr 2000, Grund et al. 2002, Storm et al. 2007).

The spatial distribution and population structure of deer in suburban, urban, and rural environments may differ. For instance, deer in suburban environments tend to have smaller home ranges compared to those in exurban areas and rural deer have the largest home ranges (Storm et al. 2007). Home ranges vary in size based on the availability of resources and competition for them. Storm et al. (2007) noted that the size of the home ranges for deer increase as the distance from the suburban areas increases. This shows that deer utilize a smaller portion of cities to fulfill their habitat requirements (Grund et al. 2002). The habitat suitability requirements for white-tailed deer change throughout the year. They need quality food sources that can be digested and those food sources change depending on time of the year, thermal cover, hiding cover, water sources. Within cities, these needs are met through residential gardens, woodlots, undeveloped properties, and buildings (Grund et al. 2002). Deer in rural and exurban environments may have to travel longer distances to find the resources they need or utilize different habitat patches at different times of the year.

Due to the smaller home ranges, there is an increased risk of higher densities of deer within the green spaces of cities. Increased densities can lead to more human and wildlife interactions or the spread of diseases. Green spaces provide unique opportunities for residents in

urban areas to interact with wildlife. Positive human-wildlife interactions include wildlife viewing, while negative interactions include the potential for disease spread, or the destruction of property due to home invasions (e.g., birds in vents or bats in attics) or vehicle collisions (Decker et al. 2010). A growing concern in Michigan is the spread of chronic wasting disease, bovine tuberculosis, and epizootic hemorrhagic disease. These diseases are spread through contact between deer and from the environment. The concern is that these diseases will eventually spread to humans. Overabundance also poses a threat to other wildlife that rely on the habitat patches that green spaces provide. While deer are natural components to an ecosystem an overabundance can alter the habitat in green spaces due to over browsing of vegetation, which can remove habitat that is crucial for other species to survive (Tremblay et al. 2007). For example, in west Michigan, white-tailed deer put forested areas at risk by acting as a vector for the spread of hemlock woolly adelgid (Deal 2007).

Overabundance and high densities of deer may pose a threat to local habitat, however, there may be risks to the deer themselves. It has been previously mentioned that deer are vectors of diseases which spread at a higher rate at high densities. However, in urban environments where home ranges are smaller, it is important to understand the genetic health of a deer herd. White-tailed deer form matrilineal groups based on the rose-petal hypothesis. The rose-petal hypothesis states that the more related a deer is the closer they will live together. This is not necessarily the case for male deer. Typically, juvenile males will leave the matrilineal group and seek out other habitats and to form bachelor herds with other males. The main reasons for this are maternal aggression or intraspecific competition with larger males within their neonatal home range (Long et al. 2005). In urban settings where deer have limited habitat dispersal might not occur at the same rate as in rural ecosystems. The results of a lack of dispersal would be breeding

with females that are related with the buck. If there is inbreeding, alleles can become fixed and have a higher rate of homozygosity. Increasing the rate of homozygosity can lead to deer that are genetically stunted and can increase the susceptibility to certain diseases (Decker et al. 2010). The use of landscape genetics provides researchers insight to the genetic health of a deer herd and to understand how genetics spread throughout the herd.

Purpose

The purpose of this study was to determine how white-tailed deer in the City of Grand Haven, Ferrysburg, and Spring Lake cluster within urban areas and how urban areas influence population size and structuring. Specifically, we wanted to determine a baseline population estimate for the deer herd within the study area. Deer are a charismatic mega-fauna in Michigan and the management of the herd is important to limit the spread of disease and to reduce negative wildlife impacts. Our goal was also to determine a population estimate and present these findings to City Managers so they can develop informed population management strategies.

Scope

Literature has primarily focused on the creation of a microsatellite panel for white-tailed deer and the effectiveness of these microsatellites for spatial genetic research (Anderson et al (2002) and Crawford et al. (2018)). Landscape genetics is a relatively new field that has an interdisciplinary approach which incorporates genetics and spatial analysis. Habitat use of white-tailed deer in rural settings is a highly researched topic where habitat suitability indices have been created and applied. However, deer in urban settings do not always follow the patterns that rural deer exhibit. Each urban area has different landscape compositions and social structures that can either hinder or perpetuate the growth of a deer herd. As urbanization continues to be at the forefront of human and wildlife interactions it is important to understand how deer use these

urban settings and to determine if anthropogenic means are influencing the level of structuring within the deer population causing subpopulations to form. This study expands on the literature by focusing on using non-invasive fecal collection methods for DNA extraction, creating a microsatellite panel for researchers to use, and implementing a genetic mark recapture method to determine a baseline population estimate that can be used by deer managers within the city.

Assumptions

1. We assumed that individuals in the population had an equal opportunity for fecal samples to be collected.
2. We assumed that there was no sampling bias during fecal collection
3. We assumed that the samples were sequenced in the order in which we dictated and that samples were not switched during processing.
4. The population was closed
5. The marks were not lost or missed
6. Marked individuals mixed randomly with unmarked
7. There was equal opportunity of capture
8. The mark did not change the behavior or increase the risk or mortality for the individual
9. We assumed that individuals with 90 percent matching alleles were considered the same individual.

Objectives

Our aim was to quantify a population estimate of the white-tailed deer herd within our study site and determine barriers to the movement of deer. We specifically aimed to: (1) Determine the genetic structure of the urban white-tailed deer herd in our study location; (2)

determine the sex-ratio of the overall population, and (3) provide city managers a census estimate for the population of white-tailed deer within the study area using non-invasive genetic mark-recapture methods.

Significance

There is a limited number of studies showing the relationship between urban white-tailed deer and how their landscape influences their movement patterns. Throughout urban landscapes there are often habitat patches, often regarded as green spaces, that provide the resources needed for deer survival. Each urban development has similar landcover structures which typically include green spaces, commercial and residential buildings, roads, and undeveloped areas; however, the amount and arrangement of green spaces within an urban area affects the distribution and spatial genetic structure of deer. Studies conducted in Michigan show that there tends to be a lower genetic diversity for white-tailed deer populations in urban areas (Blanchong et al. 2013). However, Crawford et al. (2018) conducted a fecal genetic study in Meridian Township Michigan where the results showed that the landscape was not a primary driver of genetic distance. This may not be the case for other urban areas of similar size and landscape structure. Therefore, prior to making decisions regarding deer management and green spaces within an urban area, it is important to understand the structure of deer populations within that area.

There is also a lack of smaller-scale genetic studies for white-tailed deer and other ungulates that are closely related. The large-scale models can look at the change in elevation and climate as driving factors for genetic distances. While this method is able to be applied for international or large-scale studies it is not applicable for localized studies. The input features for localized studies will be unique for each study location.

CHAPTER 2. GENETIC STRUCTURE OF URBAN WHITE-TAILED DEER IN WEST MICHIGAN

ABSTRACT

In urban areas, green spaces are used by humans and wildlife. The proximity between them can lead to both positive and negative interactions, which can make managing urban wildlife difficult. Managers are challenged due to conflicts between wildlife population sizes that can be naturally supported versus those that are socially tolerable. White-tailed deer (*Odocoileus virginianus*) thrive in urban environments because their habitat requirements are met within green spaces and backyard vegetation. Matrilineal groups of urban white-tailed deer live within the same areas, at times forming high densities that can lead to the spread of diseases or environmental pests, including hemlock wooly adelgid and ticks. Understanding the relatedness between those matrilineal groups may assist managers in understanding their movement patterns and help identify management strategies. Technological advancements in genetics have allowed researchers to investigate wildlife population structure at a molecular level and use that information in population management. The objective of this study was to understand the genetic structure of an urban deer population to determine baseline population data on which to make management decisions. We investigated deer population genetics from fecal samples collected throughout Grand Haven, Michigan, and the surrounding residential areas. Using microsatellites in a mark-recapture study, we assessed genetic clustering, sex ratios and calculated a population estimate. Results revealed 5 genetic clusters, a 24:1 female to male sex ratio and a population density of approximately 15 deer/ km^2 (range = 7.9–21.9 deer/km^2). Mantel tests showed a positive correlation between distance and genetic diversity within our study area. Results may be

used in future landscape genetics research to investigate potential features inhibiting or facilitating gene flow within urban areas.

KEY WORDS: genetics, microsatellites, urban, white-tailed deer

Urban areas tend to have an interspersion of green spaces, which are areas open to the public for recreational activities. These spaces range from soccer fields to forested wood lots, and have both social and ecological value (Wolch et al. 2014). Green spaces provide urban residents with opportunities for recreational activities that allow for immersion in nature and provide ecological services including storm water retention and wildlife habitat (Wolch et al. 2014, Atiqul Haq 2021). Managing urban green spaces is challenging. Managers must allow public access while also considering the biological challenges which include forest regeneration and heterogeneity of the landscape (Aronson et al. 2017), mitigating invasive plant species dispersed by humans or wildlife (Gaertner et al. 2016), and minimize negative human-wildlife interactions.

Green spaces provide residents of urban areas unique opportunities to interact with wildlife (Soulsbury and White 2015). Positive human-wildlife interactions include wildlife viewing and pollination of crops and ornamental plantings. Negative human-wildlife interactions include disease spread, the destruction of property (e.g., birds in vents or bats in attics), or vehicle collisions (Cornicelli et al. 1996, Nielsen et al. 2003, Decker et al. 2010). For example, a growing concern in Michigan is the potential for disease spread or vehicle collisions with white-tailed deer (*Odocoileus virginianus).* These risks increase with greater proximity to humans and larger population sizes as is common in urban areas. Understanding how our urban infrastructure is used by wildlife such as deer can help managers reduce the number of negative interactions while facilitating positive interactions.

White-tailed deer are highly successful in urban environments because their shelter and food requirements are met within green spaces and residential areas (Grund et al. 2002). Urban deer populations provide residents a chance to view wildlife, however, this positive outlook is not shared by every resident. Some residents perceive the overabundance of deer as an increased potential for disease spread and habitat destruction. White-tailed deer are natural components to most of the ecosystems in Michigan, however, an overabundance can alter urban green spaces. White-tailed deer form matrilineal groups in which families of deer congregate together (Cullingham et al. 2011). This means that large familial groups can over-browse vegetation in an urban green space and destroy habitat that would be used by other wildlife. Additionally, congregations of deer may increase rates of disease spread through direct contact (Blanchong et al. 2003, Tremblay et al. 2007). Furthermore, deer readily seek seasonal habitat components from residential yards and gardens within urban areas, which may either provide positive wildlife viewing opportunities for humans or negative sentiments due to perceptions of deer as a nuisance. City managers are faced with decisions on how to mitigate conflict while maintaining positive experiences between humans and wildlife. These decisions are not effectively made without knowledge of the population structure within urban areas.

Fortunately, technological advancements in genetics have allowed researchers to investigate wildlife population structure at a molecular level and use that information in population management. For example, genetic analyses can be used to estimate population size, sex ratios, relatedness and distribution of individuals and family groups within an area. This information is useful for understanding relationships between landscapes and population structure and movement patterns, which may help managers identify areas susceptible to damage or conflict caused by deer. Genetic material is readily available within ecosystems; thus, genetic

data for population genetic analyses may be acquired in a non-invasive way (Tremblay et al. 2007). For example, to determine if a species is present within an area, researchers can use environmental deoxyribonucleic acid (eDNA) which can be obtained through soil and water samples. Researchers and managers can also use DNA that is available through feces or other tissues to identify specific individuals within an area (Crawford et al. 2018). Using fecal samples, depending on the target species, may reduce the amount of sampling effort and cost during the collection of genetic material and removes the need to handle the animal. For common species feces are readily available and have the potential to give insight into what the animal is eating and what habitats they are using.

The purpose of this study was to understand the genetic structure of an urban deer population to determine baseline population data on which to make management decisions. Our objectives were 1) determine the genetic structure of urban white-tailed deer herd near Grand Haven, Michigan and 2) provide managers a population estimate of white-tailed deer within the study area using easily collected DNA from fecal samples. We predicted that there would be multiple subpopulations within the study area created by anthropogenic and natural causes. We also expected the population composition to be mostly female deer due to the social structure of deer herds (Cullingham et al. 2011).

STUDY SITE

Our study site was encompassed by the cities of Grand Haven, Ferrysburg, and Spring Lake, Michigan which totaled 65 km^2 (Figure 1) sampling occurring within 40 km^2 of the study site (Figure 2). Our study site is located in West Michigan which is 53 km west of Grand Rapids along the shore of Lake Michigan. The three cities collectively contain a mosaic of residential

and commercial developments and public green spaces. The GPS coordinates for centroid of the study area are -86.2211 W and 43.06012 N. West Michigan has been classified as a humid continental climate (Kottek et al. 2006). According to the US Climate data provided by worldclimate.com our study site has an average annual high temperature is 13 ℃ and the average low is 4 ℃. The annual precipitation (rain and snow) in is 83 cm.

Our study site is comprised of 10 major land cover classifications which are interspersed throughout the city. The land covers were bare land (3%), high intensity developed (3%), low intensity developed (16%), medium intensity developed (8%), palustrine wetlands (13%), unconsolidated shore (<1%), water (12%), forests (32%), cultivated (5%), and developed open spaces (8%) (Table 2) (National Oceanic and Atmospheric Administration 2016). Impervious surfaces include houses, parking lots and industrial sectors. The major water ways that are in the study site are Lake Michigan to the west and the Grand River to the north and east. Notable green spaces included Duncan Woods (16 ha), Mulligan's Hollow (32 ha), Rosy Mound (66 ha), Hofma Preserve (131 ha), and Kitchel Lindquist Preserve (46 ha). The forest vegetation structure was similar throughout the major preserves and parks. Deciduous forests consisted of beech (*Fagus grandifolia*) and maple (*Acer spp.*) trees dominating the canopy with oaks *(Quercus spp.*) scattered throughout. Coniferous forests were primarily dominated by red pine (*Pinus resinosa*), Scots pine (*Pinus sylvestris*) and Eastern hemlock (*Tsuga canadensis*). The main infrastructure used in Grand Haven were residential houses and commercial stores and warehouses in which the areas totaled more than 60% of the study site.

METHODS

Sample Collection and Processing

We divided the sampling area into 4 quadrants by prominent landscape features which included US-31 (east-west division) and the Grand River (north-south division; Figure 2). Within the 4 quadrants we collected deer fecal pellets from public green spaces between July 2019 and March 2020 and stored them in sterile 50 ml falcon tubes. To determine publicly- accessible green spaces, we used Ottawa County property mapping provided by gis.miottawa.org (Ottawa County Geospatial Insights and Solutions). We collected samples in green spaces by walking around for 2 hours following game trails and locating bedding areas, and by walking randomly. We collected the fecal samples from individual pellet groups only if they passed an initial quality check. For instance, pellets could not be dried out, covered in sand, and they needed to be fully intact. To avoid DNA degradation samples were not collected during rain or snowfall. When sampling in the summer, as sunlight can cause DNA degradation, only fresh samples were collected that had a visible mucosal membrane. Since our study site has sandy soils and dune systems, extra precautions were taken while sampling in these systems to avoid collecting feces with excess sand because abrasive sediments can break down the mucosal membrane of the feces and remove DNA. GPS coordinates were taken at each fecal sample location to allow for future spatial analysis. The majority of the samples were swabbed in the field using a histobrush dipped in cell lysis buffer (ZymoResearch, Irvine, California) (Ramon-Laca et al. 2015) after we determined swabbing was effective on fresh samples. The samples were then transported to the lab on ice for further processing.

We processed 372 fecal samples following the protocol designed by ZymoResearch for the Quick-DNA Miniprep Plus Kit (ZymoResearch). We suspended the histobrush in a solution

that contained 20 μl of proteinase K 20 mg/mL, 200 μl of elution buffer and 200 μl of a cell buffer in a microcentrifuge tube. We placed the microcentrifuge tube in a 55 ℃ water bath to incubate for 10 minutes then removed the brush and added one volume of genomic binding buffer. We added the sample to a spin column and ran the sample in the centrifuge for one minute at 12,000 x g. Keeping the speed and time consistent, we added 400 μl of DNA prewash and repeated the centrifuge process. Once the DNA prewash was discarded, we centrifuged two more times using 700 μl and 200 μl g-DNA wash buffer, respectively. We then eluted the DNA in two consecutive centrifuge processes. For the elution process we added 30 μl of elution buffer that was incubated at 55℃ for 5 minutes and then centrifuged the sample. Once the first spin was done, we added 30 μl more of elution buffer and centrifuged the sample one last time giving us 60 μl of eluted DNA.

To create a positive control, we collected both male and female white-tailed deer heads from local deer processors. We dissected the heads and removed tissue from the masseter muscle and removed the lymph nodes from the throat. Once they were harvested, we stored samples on ice and transported them to -80 ℃ freezers. After the samples were completely frozen, we pulverized them into a fine dust using a mortar and pestle. To keep the samples frozen during the pulverization process we kept the mortar on dry ice while pouring liquid nitrogen over the tissue. Once the sample was fully pulverized, we collected the powder and stored it in a 50 ml falcon tube at -80℃ until it was processed into eluted DNA following the protocol from Baise et al. (2002).

Sex Determination

To determine the sex of the sample we ran polymerase chain reactions (PCR) using the zinc-finger intron as well as the SRY (sex-determining region of the Y-chromosome) intron (Lindsay

and Belant 2008). The zinc-finger intron has amplicon size variation between the X (236 base pairs) and Y (417 base pairs) locus which can be determined by running samples on an agarose gel during gel electrophoresis (Cathey et al. 1998, Lindsay and Belant 2008). [Note: we used reported primers from Lindsay and Belant (2008)]. The size difference between the introns allowed us to determine if one gene was present or if both were present. The SRY intron is male-specific and produces a band if the sample had male DNA and if there was not a band present then we assumed it was a female. However, it is possible that no band could have also indicated a failed PCR reaction. If a sample generated a male-specific PCR product of the expected size using the primers for the zinc finger intron, it was verified to be a male sample using the SRY primer.

Microsatellite Panel

We chose a subset of 10 autosomal microsatellite loci from a larger microsatellite panel published by Anderson et al. (2002), Crawford et al (2018), and Miller et al. (2019) (Table 1) for our population genetic analysis. The selected loci from the panel were chosen to maximize allelic variation. A higher number of alleles means that there is a greater chance of heterozygosity in a population which will help reduce the number of shared alleles, and would help with population genetics and genetic mark-recapture analysis. When using polymorphic microsatellites, the greater number of samples increases the precision of the genetic tests (Kalinowski 2004). Our PCR master mix included 12.5 µl Master Mix 2x (from Promega Corporation), 0.5 µl Forward Primer 250 uM, 0.5 µl Reverse Primer 250 uM, 10 µl DNA, 1.5 µl nuclease free water, 1 µl BSA and the steps for our PCR process were as follows: Initialization for 10 minutes at 95 °C, denaturation for 30 seconds at 95 °C, annealing at the specific temperatures (i.e., Table 1) for 30 seconds, elongation at 72 °C for 1 minute, steps 2–4 were repeated 38 times, then lastly there

was a final elongation at 72 °C for 5 minutes. We used the male muscle DNA as our positive control and nuclease free water as our negative control. We ran each microsatellite in individual PCR reactions and then pooled the microsatellite amplicons into groups of five, based on previously published amplicon size and fluorescent tag (Table 1), for fragment analysis using ABIs Genetic Analyzers 3730xl with the liz500 size standard. To determine the amplicon size for the microsatellites we used PeakScanner v 2.0 (Applied Biosystems Inc., Foster City, California) and compared them using the base pair range provided in Anderson et al. (2002) and Crawford et al. (2018). The samples must have contained alleles for six of the microsatellites to have been used in the population estimate and genetic tests.

Population Estimates and Structure

To determine the identity of individuals, we used CERVUS 3.0.7 (Field Genetics, London, United Kingdom). For identity analysis CERVUS compares the genotype of one sample to that of all others. If there was a direct match, then it was recorded as a recapture. CERVUS also used fuzzy matching which allows researchers to set the desired number of matching genotypes for positive recaptures for their population estimate. Individuals needed to match 9 of the microsatellites on separate days to be determined recaptures. We separated the samples into 2 time periods, summer (July through September 2019) and fall/winter (October through March 2020). The number of repeat captures during the second time period were then used in the Lincoln-Peterson mark-recapture model to determine a population estimate. We used the equation:

$$\widehat{N} = \frac{MC}{R}$$

where,

$\widehat{N}$ = population size estimate

M = total number of marked individuals during the first time period

C = total number of individuals captured during the second time period

R = number of recaptured individuals during the second time period

We also computed the standard deviation ($\hat{S}$) and 95% confidence limit ($N \pm 1.96 * \hat{S}$) using the equation:

$$\hat{S} = \sqrt{\frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(R+2)}}$$

Lincoln-Peterson mark-recapture model has five assumptions (Seber 1982): 1) the population is closed, 2) the marks cannot be lost or missed. 3) marked individuals mix randomly with the unmarked individuals, 4) there is an equal opportunity of capture, and 5) the mark does not change the behavior or increase the risk of mortality for the individual. The overall inbreeding coefficient (F_{IS}) and estimates of Hardy Weinberg Equilibrium were calculated in GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). To determine the number of subpopulations within our study site we used discriminate analysis of principal components (DAPC) (Jombart et al. 2010). We also ran the DAPC analysis using only the females from 148-samples (i.e., that amplified at a minimum of 6 microsatellites) to determine if females would have different clustering patterns without the males considering they stay in matriarchal groups. To determine the number of populations we used the lowest Bayesian information criterion (BIC). We visualized the membership of individuals in each group using an assignment plot.

RESULTS

Throughout the study, we collected 372 samples containing at least 8 fecal pellets. Most of the samples were collected in locations classified as forested or developed land (Table 3). We used a subset of 148 samples that amplified at a minimum 6 microsatellites.

We determined a sex ratio for our study area using the proportion of females to male samples collected. We were able to identify a female to male sex ratio of 24:1 using the 148-sample subset. When amplifying the 10 microsatellites for our 148 samples, the amplification success rate for the microsatellites were 90%, meaning we were only missing alleles for 10% or less for all of the samples at that microsatellite locus. In the collection of 148 samples used in our population analyses, each microsatellite could be amplified successfully and identified in greater than 90% of individuals.

The result from the isolation by distance test was not significant (p=0.598), suggesting that distance may not be the only force acting on spatial variation in allele frequencies across our sampled deer population. Using the spatial principal components analysis (sPCA) and Monte-Carlo to test if there was genetic structuring within the study area we determined that our global p-value was 0.001, indicating there was some spatial variation among the deer groups.

During the first sampling period (i.e., July–September 2019), we captured and marked 85 individuals. During the second sampling period (i.e., October–March 2020), we captured 63 individuals, 9 of which were recaptures. The 9 recaptures were identified based on the stipulation that recaptures must match 90% of alleles from individuals captured during the first time period. We used the Lincoln-Peterson's method with these 9 recaptures to calculate a population estimate for our study area. The population estimate for the study area was 595 individuals which

equated to a density of 15 deer/km^2 (38 deer/mi^2) and the 95% confidence intervals ranged from 314–876 individuals.

The results of the DAPC analysis showed that there was a potential of 5 genetic clusters within the study area with a BIC value of 185.5 (Figures 4, 5). When using the subset of only females, we still had 5 genetic clusters throughout our sampling location (Figures 6, 7). The DAPC analysis revealed that deer in the northeastern section of the study area were the most genetically distinct group from the other clusters based on posterior membership probability for all deer (Figure 5) and the female only subset (Figure 7). In the dataset, not all allele frequencies aligned with Hardy-Weinberg equilibrium, likely because we sampled related individual that were part of matriarchal groups. The inbreeding index (F_{IS}) was approximately 0.23, which is supporting evidence that the samples collected likely consisted of family groups.

DISCUSSION

Our study aimed to assess urban deer population size, genetic population structure, and sex ratios using easily accessible DNA from deer feces. Using fecal analysis and microsatellites, we were able to gain insight into how many potential genetic clusters were present within our sampling area and we were able to estimate a population size and density for our sampling location. There was not a difference in the number of clusters between the data subsets that contained females and males compared to the dataset with only females. This could potentially be from the low number of males (n = 6) that successfully amplified at 6 or more microsatellite locations, and thus, even the DAPC analysis for the entire dataset is likely being driven by the female population structure. A larger number of males in the subset may change the number of clusters within the study area. White-tailed deer, like elephants, form groups revolving around the

females of the herd (i.e., matrilineal groups). The deer within these groups have increased relatedness to one another compared to deer found in different geographic regions. Different factors influence the proximity of these matrilineal groups to one another. These factors can be natural, man-made, or a combination of both. Our results from isolation by distance show that there is a potential that something other than geographic distance between groups is causing the genetic differences between subpopulations (Athanasiadis and Moral 2013). Future studies can be used to determine what factors are causing genetic differences among groups. Peterman (2018) developed a software called ResistanceGA that models resistance values of landscape features to determine how easily genes are able to move through areas dominated by specific landcover classifications.

The distribution of urban deer herds fluctuates at different times of year depending on which life requisites need to be met and how they are being met (Grund et al. 2002). The seasonal shift in habitat requirements may influence the structuring that occurs with urban deer populations. For instance, within our study area fecal samples collected throughout the spring, summer and fall were found in varying quantities in different vegetation types (Table 3). During the fall deer tend to use areas producing hard mast (Nixon et al. 1970), which include most of the woodlots within the study area. Without pressure to leave, the deer will become resident in these woodlots and start to isolate from the rest of the population which was evident based on the genetic clustering occurring in the southwestern region of our study area (Figure 5). During the spring and summer, most samples were found in developed and cultivated areas such as parks and fields, which provide necessary food and cover during that season (Rogers et al. 1981). In urban environments deer move between habitat patches when different life requisites need to be met (Kilpatrick and Spohr 2000). Within the northwest, southwest, and southeast sections of the

study area we saw higher levels of admixture compared to those within the northeastern section. The increase level of admixture may be influenced by the ability for deer to move and interact within areas of suitable habitat patches by using various corridors. For example, matrilineal groups have the potential to overlap in areas that produce suitable habitat, such as open lawns and gardens adjacent to small woodlots in residential areas. The movement through residential areas may strain the relationship between stakeholders and deer due to browsing of gardens and ornamental plantings (DeStafano and DeGraff 2003). In cases where habitat needs can be met in smaller habitat patches, family groups may separate from other family groups, which can create clusters within the population. Based on the conditions and the availability of different habitat types, the deer population in Grand Haven likely follow this trend based on the level of admixture within the study area.

White-tailed deer will adjust their home ranges as a response to environmental pressures such as flooding and larger quantities of snow (Grund et al. 2002); both of which occurred during the sampling sessions. For instance, in the northeastern portion of the study area, the green spaces were restricted by surrounding floodwaters, as they were in the Grand River floodplain. In the agricultural and forested regions along the eastern boundary of the study site, deer may have larger home ranges during the nongrowing season compared to the growing season (Walter et al. 2009). During the growing season corn and blueberry fields may provide cover and food sources reducing the need to expand their home ranges. As the crops are harvested the deer may be moving into forested areas in search of forage (Walter et al. 2009). The lack of resources at different times of the year may cause the familial clusters of white-tailed deer to overlap creating a new breeding potential within a location allowing genes from two groups to become integrated. We can see this trend occur within Grand Haven based on the high

levels of admixture. In contrast, the northeast portion of our study area displayed low amounts of admixture with the other sampling units. The Grand River split our study area into the North and South units. The river itself could be acting as a barrier to movement (Blanchong et al. 2008) of the deer in the Northeast limiting gene flow into the area from the rest of the study site. Alternatively, the decreased admixture could be from sampling bias since flooding events in the northeast section of our study area limited the number of green spaces that could be sampled. The fecal samples collected are likely from related individuals within a family group. A landscape-genetics analysis would potentially reveal whether or not landscape features are affecting admixture in this area (Peterman et al. 2014).

We calculated a high density of deer within our study area, but comparable to densities found within other urban areas in the Midwest (McAninch et al. 1993, DeNicola et al. 2008). Deer at high densities may cause negative human-wildlife interactions such as vehicle-deer collisions and over browsing of ornamental plantings as a supplementary food source. At higher densities of deer, we speculate that US-31 may have increased cases of deer collisions which will result is thousands of dollars in repairs. At high densities, deer are also more susceptible to disease transmission such as chronic wasting disease and bovine tuberculosis. Competition for resources would also threaten the deer within the study site if densities become unsustainable by the habitat patches.

White-tailed deer are predominately male-dispersed, meaning that males will be forced out of a family group or a location by a male competing for resources or mates, or the matriarch of the familial group (Hawkins et al. 1971, Greenwood 1980, Kie and Bowyer 1999). This type of dispersal often leads to a higher density of females which tend to move less compared to males (McCance and Baydack 2018). The size of matrilineal groups is influenced by how well

the life requisites of deer are met within a landscape (Cullingham et al. 2011). Without natural or anthropogenic influences, the success rate of local geographic and genetic dispersal will increase which will increase the likelihood of population growth and sustainability (Long et al. 2005), especially if local populations are largely female (Blanchong et al. 2013), as in the case in this study indicated by a sex ratio being at least 24:1 female to male (Blanchong et al. 2013). Typically, in urban areas the ratio will range from 2:1 to 6:1 female to male (DeNicola et al. 2008). Our high female to male ratio may be caused by the level of male dispersal in late fall throughout the summer or there may not be many resident males within our study site. There is also a possibility that individuals may have been sexed incorrectly due to amplification errors during the PCR process. Furthermore, there is a chance that the collection of fecal samples could have introduced sampling bias into the study. If a large group of females frequented an area that was being sampled it is more likely that fecal pellets from those individuals will be collected at that location within the sampling timeframe.

Understanding the size of a population is imperative for establishing a baseline for management decisions. Mark-recapture methods provide researchers an estimation of population size within a study area, but in some cases, it is difficult to capture individuals, especially when the species that are being studied occupy a large range or are illusive in nature (Bellemain et al. 2005). To combat the issue of illusiveness and large ranges, researchers can use a genetic mark-recapture study using DNA sampled from fecal material, where replicating the microsatellite amplification and calling allow researchers to develop a more accurate population estimate. In this study, fecal samples allowed us to cut cost and sample the study area using efficient methods. Using fecal samples allows researchers to gain insight into how urban deer herds interact with each other and their environment without harming or handling the animals.

For our study we had 9 recaptures using the 148 samples that met the requirements for 90% matching allele analysis. Based on these recapture estimates, we estimated the Grand Haven and surrounding area population to be 595 individuals (15 deer/km^2, range ≈ 8 – 22 deer/km^2). However, this density may be an underestimate of the true population size and density within the study area. As the method becomes more refined and as microsatellite analysis is replicated at a larger scale it is likely that the estimate will become more accurate since individuals can be identified with increased certainty. The population may not have been truly closed for this study. Without spatial analyses we are not certain how much of the study area is being used by deer. The movement of deer at different times of the year will potentially bias the density estimates for the study area. Continued sampling over multiple years may address the issue of temporal uses of the landscape. Without understanding landscape resistance and areas not being utilized by the deer we speculated that the functional (usable) area was approximately 40 km^2. Using more samples for the mark-recapture would allow for a more accurate estimation of population size. McAninch et al. (1993) reported densities ranging from 15 – 70 deer/km^2 in urban areas, which corresponds to our density estimate (i.e., 15 deer/km^2). It is important to note that this density was still more than double the density that the Michigan Department of Natural Resources had determined as the population management goal within Ottawa County where Grand Haven is located (6.69 deer/km^2; 2003 estimate from the DNR).

When utilizing genetic mark recapture studies it is important to understand that samples must be independent from another. Fecal samples collected on the same day from the same location cannot be considered independent and need to be treated as one sample collection. Software such as Cervus 3.0.7 (Field Genetics, London, United Kingdom) allows for individual

identification, however the software does not separate samples by day and recaptures should be verified when using this method.

While fecal sampling for mark-recapture analysis to estimate population density is a non-invasive sampling method, there are challenges associated with these approaches. Environmental factors, such as temperature or UV radiation, may have limited the yield of DNA available in the mucosal membrane. Abrasive substances collected with the fecal material, such as sand, may compromise the membrane reducing the DNA available. For future studies, the samples should undergo a second PCR amplification to verify results of DNA calling, and the study size should be expanded to determine if the population is moving from outside of the study area.

In future studies, a more reliable population estimate may be obtained from other sources of DNA including blood or tissue samples from a deceased or captured and sedated deer. These tissues may also be used for other studies including disease identification (Lang and Blanchong 2011). Other tissues yield a high concentration of DNA which we observed when using the non-fatty masseter muscle tissue for our positive control (Pease et al. 2009). When using feces as the non-invasive source of DNA the variability of potency and contamination levels is increased. It is beneficial to collect feces shortly after excretion when the mucosal membrane is intact and not degraded, thus decreasing the chance of having alleles drop out during PCR and having False alleles. These missing alleles may lead to misidentifying individuals and skewing a population estimate (Huber et al. 2002). If the inner part of the pellet is included in the DNA extraction the proportion of successful PCR reaction would also likely decline (Wehausen et al. 2004). Taberlet et al. (1996) noted that if the DNA is not 99% reliable then replicate PCR reactions are necessary to confirm individual genotypes. Due to monetary constraints, we were not able to fully replicate our samples. However, we did reamplify some samples when the initial PCR reaction failed.

This reamplification increased the success rate for our microsatellites from 37% to 90%. Thus, this research show the potential use of non-invasive genetic sampling for population assessment, yet care must be given when interpreting the results considering the caveats associated with DNA quality from fecal material.

MANAGEMENT IMPLICATIONS

Genetic analysis is a useful tool for mark recapture studies because DNA in the form of fecal samples can be found everywhere and there is little to no contact with the species being studied. This non-invasive method can be beneficial in urban settings where it is challenging or infeasible to handle animals directly. Using a non-invasive genetics approach allows managers to receive valuable data for population management without handling white-tailed deer (Tremblay et al. 2007). Genetic studies allow managers to understand the sex ratio of a population, where in some cases like white-tailed deer is incredibly influential on the densities of matrilineal groups. Managers have a difficult time managing in urban environments due to stakeholder perceptions and the lack of knowledge of the population size of the local deer herd. Genetic mark recapture studies allow managers a cost-effective way to determine a baseline estimate for their deer herds.

In 2008, Grand Haven developed an urban deer management plan (City of Grand Haven 2008). At the time, deer population estimates ranged from 11 deer/km^2 (30/mi^2) throughout Ottawa County (2003 estimate from the DNR), with a DNR population management goal of 6.69 deer/km^2 (17 deer/mi^2). Based on our study, currently, the city of Grand Haven holds a deer population higher than the estimate throughout the county, and more than twice what the DNR recommended for the county. Additionally, the sex ratio is out of balance, with more than 24 does per 1 buck within the city. This study allows managers in Grand Haven to have a working

and current population estimate, sex-ratio, and population genetic structure revealing potential clustering of deer. This information may help to focus management strategies within ecologically defined management units (Cornicelli et al. 1996). Results of this study should be used as baseline data to establish monitoring and management plans for the urban deer herd.

ACKNOWLEDGEMENTS

We would like to thank the Michigan Space Grant Consortium and Grand Valley State University for providing funding for this project. We would also like to thank J. Veldboom and A. Patil for helping with the sample processing. We would also like to thank the reviewers for their time and comments to help improve this manuscript.

LITERATURE CITED

Anderson, J. D., R. L. Honeycutt, R. A. Gonzales, K. L. Gee, L. C. Skow, R. L. Gallagher, D. A. Honeycutt, and R. W. DeYoung. 2002. Development of Microsatellite DNA Markers for the Automated Genetic Characterization of White-Tailed Deer Populations. The Journal of Wildlife Management 66:67–74.

Athanasiadis, G., and P. Moral. 2013. Spatial principal component analysis points at global genetic structure in the Western Mediterranean. Journal of Human Genetics 58:762–765.

Atiqul Haq, S. M. D. 2021. Urban Green Spaces and an Integrative Approach to Sustainable Environment. Pages 147–162 in. URBAN ECOLOGY: strategies for green infrastructure and land use. essay, *APPLE ACADEMIC PRESS*, S.l.

Aronson, M. F., C. A., Lepczyk, K. L., Evans, M. A., Goddard, S. B., Lerman, J. S., MacIvor, C. H., Nilon, T., Vargo. (2017). Biodiversity in the city: Key challenges for urban green space management. *Frontiers in Ecology and the Environment*, 15(4), 189–196.

Biase, F. H., M. M. Franco, L. R. Goulart, and R. C. Antunes. 2002. Protocol for extraction of genomic DNA from swine solid tissues. Genetics and Molecular Biology 25:313–315.

Bellemain, E., J. E. Swenson, D. Tallmon, S. Brunberg, and P. Taberlet. 2005. Estimating Population Size of Elusive Animals with DNA from Hunter-Collected Feces: Four Methods for Brown Bears. Conservation Biology 19:150–161.

Blanchong, J. A., A. B. Sorin, and K. T. Scribner. n.d. Genetic diversity and population structure in urban white-tailed deer. 2013. The Journal of Wildlife Management - Wiley Online Library. <https://wildlife.onlinelibrary.wiley.com/doi/full/10.1002/jwmg.521>. Accessed 29 Aug 2019.

Cathey, J. C., J. W. Bickham, and J. C. Patton. 1998. Introgressive Hybridization and Nonconcordant Evolutionary History of Maternal and Paternal Lineages in North American Deer. Evolution 52:1224–1229.

City of Grand Haven. 2008. City of Grand Haven Urban Deer Management Plan March 2008.

Cornicelli, L., A. Woolf, and J. L. Roseberry. 1996. White-Tailed Deer Use of a Suburban Environment in Southern Illinois. Transactions of the Illinois State Academy of Science 89:93-103.

Crawford, J. C., A. D. Quinn, D. M. Williams, B. A. Rudolph, K. T. Scribner, and W. F. Porter. 2018. Fine-scale spatial genetic structure of deer in a suburban landscape. The Journal of Wildlife Management 82:596–607.

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

Decker, D. J., D. T. N. Evensen, W. F. Siemer, K. M. Leong, S. J. Riley, M. A. Wild, K. T. Castle, and C. L. Higgins. 2010. Understanding Risk Perceptions to Enhance Communication about Human-Wildlife Interactions and the Impacts of Zoonotic Disease. ILAR Journal 51:255–261.

DeNicola, A. J., D. R. Etter, and T. Almendinger. 2008. Demographics of non-hunted white-tailed deer populations in suburban areas. Human–Wildlife Interactions. 70.

DeStefano, S., and R. M. DeGraaf. 2003. Exploring the Ecology of Suburban Wildlife. Frontiers in Ecology and the Environment 1:95–101.

Gaertner, M., Larson, B. M. H., Irlich, U. M., Holmes, P. M., Stafford, L., Wilgen, B. W. van, & Richardson, D. M. 2016. Managing invasive species in cities: A framework from Cape Town, South Africa. Landscape and Urban Planning, 151:1–9.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28:1140–1162.

Grund, M. D., J. B. McAninch, and E. P. Wiggers. 2002. Seasonal Movements and Habitat Use of Female White-Tailed Deer Associated with an Urban Park. The Journal of Wildlife Management 66:123–130.

Hawkins, R. E., W. D. Klimstra, and D. C. Autry. 1971. Dispersal of Deer from Crab Orchard National Wildlife Refuge. The Journal of Wildlife Management 35:216–220.

Huber, S., Bruns, U., and Arnold, W. 2002. Sex determination of red deer using polymrease chain reaction of DNA from feces. Wildlife society bulletin 30(1): 208-212.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Kalinowski, S. T. 2004. Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94:33–36.

Kie, J. G., and R. T. Bowyer. 1999. Sexual Segregation in White-Tailed Deer: Density-Dependent Changes in Use of Space, Habitat Selection, and Dietary Niche. Journal of Mammalogy 80:1004–1020.

Kilpatrick, H. J., and S. M. Spohr. 2000. Movements of Female White-Tailed Deer in a Suburban Landscape: A Management Perspective. Wildlife Society Bulletin (1973-2006) 28:1038–1045.

Kilpatrick, H. J., and S. M. Spohr. 2000. Spatial and Temporal Use of a Suburban Landscape by Female White-Tailed Deer. Wildlife Society Bulletin (1973-2006) 28:1023–1029.

Kohn, M. H., E. C. York, D. A. Kamradt, G. Haught, R. M. Sauvajot, and R. K. Wayne. 1999. Estimating population size by genotyping faeces. Proceedings of the Royal Society B: Biological Sciences 266:657–663.

Kottek, M., J. Grieser, C. Beck, B. Rudolf, and F. Rubel, 2006: World Map of the Köppen-Geiger climate classification updated. Meteorol. Z., 15, 259-263.

Lang, K. R., and J. A. Blanchong. 2012. Population genetic structure of white-tailed deer: Understanding risk of chronic wasting disease spread. The Journal of Wildlife Management 76:832–840.

Lindsay, A. R., and J. L. Belant. 2008. A simple and improved PCR-based technique for white-tailed deer (Odocoileus virginianus) sex identification. Conservation Genetics 9:443–447.

Long, E. S., D. R. Diefenbach, C. S. Rosenberry, B. D. Wallingford, and M. D. Grund. 2005. FOREST COVER INFLUENCES DISPERSAL DISTANCE OF WHITE-TAILED DEER. Journal of Mammalogy 86:623–629.

McAninch, J. B., L. P. Hansen, and K. A. Kelly. 1993 Urban Deer: A Manageable Resource? 55th Midwest Fish and Wildlife Conference, St. Louis, Missouri.

McCance, E., and R. K. Baydack. 2018. Spatial and Temporal Use of an Urban Landscape by White-Tailed Deer. Environmental Analysis & Ecology Studies 3:1–15.

Miller, W. L., J. Edson, P. Pietrandrea, C. Miller-Butterworth, and W. D. Walter. 2019. Identification and evaluation of a core microsatellite panel for use in white-tailed deer (*Odocoileus virgirginianus*). BMC Genetics. https://doi.org/10.1186/s12863-019-0750-z

National Oceanic and Atmospheric Administration, Office for Coastal Management. 2016. "C-CAP Regional Land Cover and Change" Coastal Change Analysis Program (C-CAP) Regional Land Cover. Charleston, SC: NOAA Office for Coastal Management. Accessed May 2021 at www.coast.noaa.gov/htdata/raster1/landcover/bulkdownload/30m_lc/.

Nielsen, C. K., R. G. Anderson, and M. D. Grund. 2003. Landscape Influences on Deer-Vehicle Accident Areas in an Urban Environment. The Journal of Wildlife Management 67:46–51.

Nixon, C. M., M. W. McClain, and K. R. Russell. 1970. Deer Food Habits and Range Characteristics in Ohio. The Journal of Wildlife Management 34:870–886.

Peakall, R. and Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Peakall, R. and Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537-2539.

Pease, K. M., A. H. Freedman, J. P. Pollinger, J. E. Mccormack, W. Buermann, J. Rodzen, J. Banks, E. Meredith, V. C. Bleich, R. J. Schaefer, K. Jones, and R. K. Wayne. 2009. Landscape genetics of California mule deer (Odocoileus hemionus): the roles of ecological and historical factors in generating differentiation. Molecular Ecology 18:1848–1862.

Peterman, W.E., G.M. Connette, R.D. Semlitsch, and L.S. Eggert. 2014. Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. Molecular Ecology, 23:2402–2413.

Peterman, W. E. 2018. ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. Methods in Ecology and Evolution 9:1638–1647.

Ramón-Laca, A., L. Soriano, D. Gleeson, and J. A. Godoy. 2015. A simple and effective method for obtaining mammal DNA from faeces. Wildlife Biology 21:195–203. Nordic Board for Wildlife Research.

Rogers, L.L., J.J. Mooty, and D. Dawson. 1981. Foods of white-tailed deer in the Upper Great Lakes Region—a review. USDA For. Serv. Gen. Tech. Rep. NC-65. 24pp.

Seber, G. A. F. (1982). The estimation of animal abundance and related parameters.

Soulsbury, C. D., and P. C. L. White. 2015. Human–wildlife interactions in urban areas: a review of conflicts, benefits and opportunities. Wildlife Research 42:541.

Storm, D. J., C. K. Nielsen, E. M. Schauber, and A. Woolf. 2007. Space Use and Survival of White-Tailed Deer in an Exurban Landscape. The Journal of Wildlife Management 71:1170–1176.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189–3194.

Tremblay, J.-P., J. Huot, and F. Potvin. 2007. Density-related effects of deer browsing on the regeneration dynamics of boreal forests. Journal of Applied Ecology 44:552–562.

Walter, W. D., K. C. VerCauteren, H. Campa, W. R. Clark, J. W. Fischer, S. E. Hygnstrom, N. E. Mathews, C. K. Nielsen, E. M. Schauber, T. R. Van Deelen, and S. R. Winterstein. 2009. Regional assessment on influence of landscape configuration and connectivity on range size of white-tailed deer. Landscape Ecology 24:1405–1420.

Wehausen, J. D., R. R. Ramey II, and C. W. Epps. 2004. Experiments in DNA Extraction and PCR Amplification from Bighorn Sheep Feces: the Importance of DNA Extraction Method. Journal of Heredity 95:503–509.

Wolch, J. R., J. Byrne, and J. P. Newell. 2014. Urban green space, public health, and environmental justice: The challenge of making cities 'just green enough.' Landscape and Urban Planning 125:234–244.

Figure Captions

Figure 1. Study area boundary (65 km^2) encompassing the city of Grand Haven, Michigan (focal city), with Ferrysburg and Spring Lake to the north.

Figure 2. Locations of white-tailed deer fecal pellets collected during 2020-2021 for a population-genetics study. Pellet collection locations were stratified relative to US Highway 31 (east-west division) and The Grand River (north-south division).

Figure 3. Land cover composition within 65 km^2 study area in west Michigan. Land cover categories were based on C-CAP 2016 data.

Figure 4. BIC values for the number of potential subpopulations with the male and female subsets of white-tailed deer near Grand Haven, Michigan (2021).

Figure 5. An assignment plot based on the level of K determined by running DAPC for male and female white-tailed deer near Grand Haven, Michigan (2021).

Figure 6. BIC values for the number of potential subpopulations of white-tailed deer near Grand Haven, Michigan (2021) based on only females from the 148-sample subset.

Figure 7. An assignment plot for female white-tailed deer in urban areas near Grand Haven, Michigan (2021) based on the number of subpopulations after running the DAPC.

Table 1: Microsatellite panels for white-tailed deer used for this research based on previous research conducted by Crawford et al. (2018) and Anderson et al. (2002). Each microsatellite fluorescently-labeled microsatellite was placed into groups based on the tags and amplicon size range. The annealing temperature for our polymerase chain reactions are listed.

Microsatellites Primers				
	Microsatellites	Fluorescent Tag	Amplicon Size	Annealing Temperature (Celsius)
Group 1	Cervid1[a]	FAM	162-196	57
	N[a]	FAM	288-336	57
	OarFCB193[a]	NED	96-124	55
	IGF1[b]	FAM	106-151	49
	Q[a]	PET	233-281	48
Group 2	RT9[b]	PET	101-127	49
	BM4107	VIC	157-181	52
	RT7	VIC	207-243	50
	BL42[b]	FAM	233-266	49
	Obcam[b]	PET	184-220	48

[a]Anderson et al. 2002
[b]Crawford et al. 2018

Table 2: Landcover categories from C-CAP 2016 data within a 65 km^2 area including the cities of Grand Haven, Ferrysburg, and Spring Lake, Michigan. Each landcover has the percent area and the total area in hectares reported along with a description of the features within the cover type.

Major Landcovers Classified From C-CAP			
Landcover	Area (ha)	Percent Area	Description
Bare Land	332	3	Sand dunes, bedrock, gravel pits
High Intensity Developed	330	3	Significant area covered by concrete and asphalt and other constructed material, <20% vegetation
Low Intensity Developed	1526	16	Contains areas of vegetation and constructed material which covers 21-49% of landscape
Medium Intensity Developed	786	8	50-79% constructed materials, typically single-family households
Palustrine Wetlands	1296	13	Tidal and non-tidal wetlands ranging from emergent wetlands to scrub-shrub
Unconsolidated Shore	1	<1	Areas of silt and sand that is subject to redistribution by water bodies, lack vegetation
Water	1141	12	Areas of open water
Forests	3128	32	Coniferous, deciduous forests, mixed forests
Cultivated	486	5	Contains areas of annual crop vegetation, pastures, livestock grazing land
Developed Open Space	787	8	Areas with constructed materials but mostly low-lying vegetation, used for recreation

Table 3: Number of white-tailed deer fecal samples collected by season (2020-2021) within predominant land cover types in a 65 km^2 area including the cities of Grand Haven, Ferrysburg, and Spring Lake, Michigan.

Number of samples collected in each major landcover		
Landcover Type	Fall/winter	Spring/Summer
Forested	52	31
Wetland	8	0
Cultivated	15	9
Developed areas	10	23

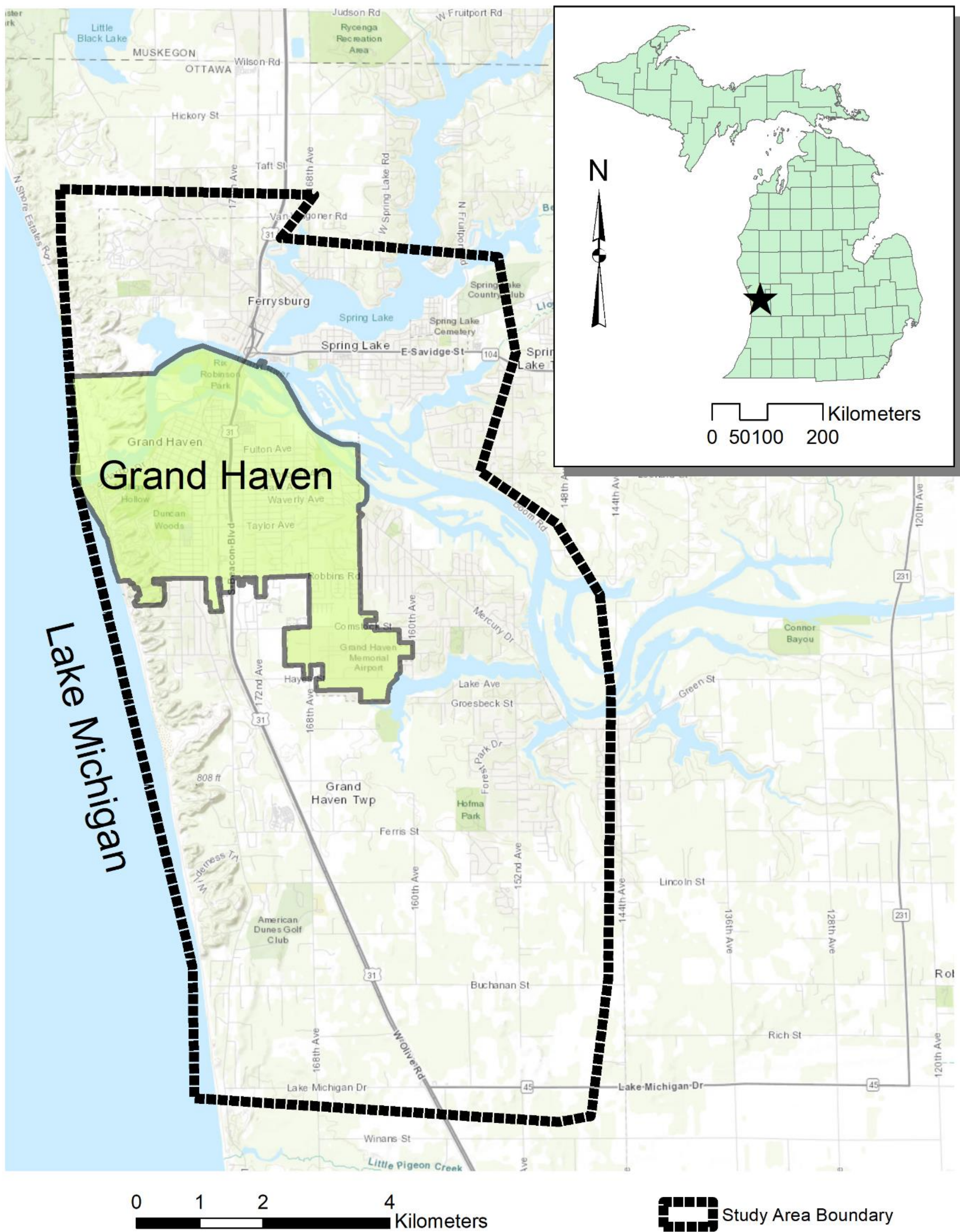

Figure 1

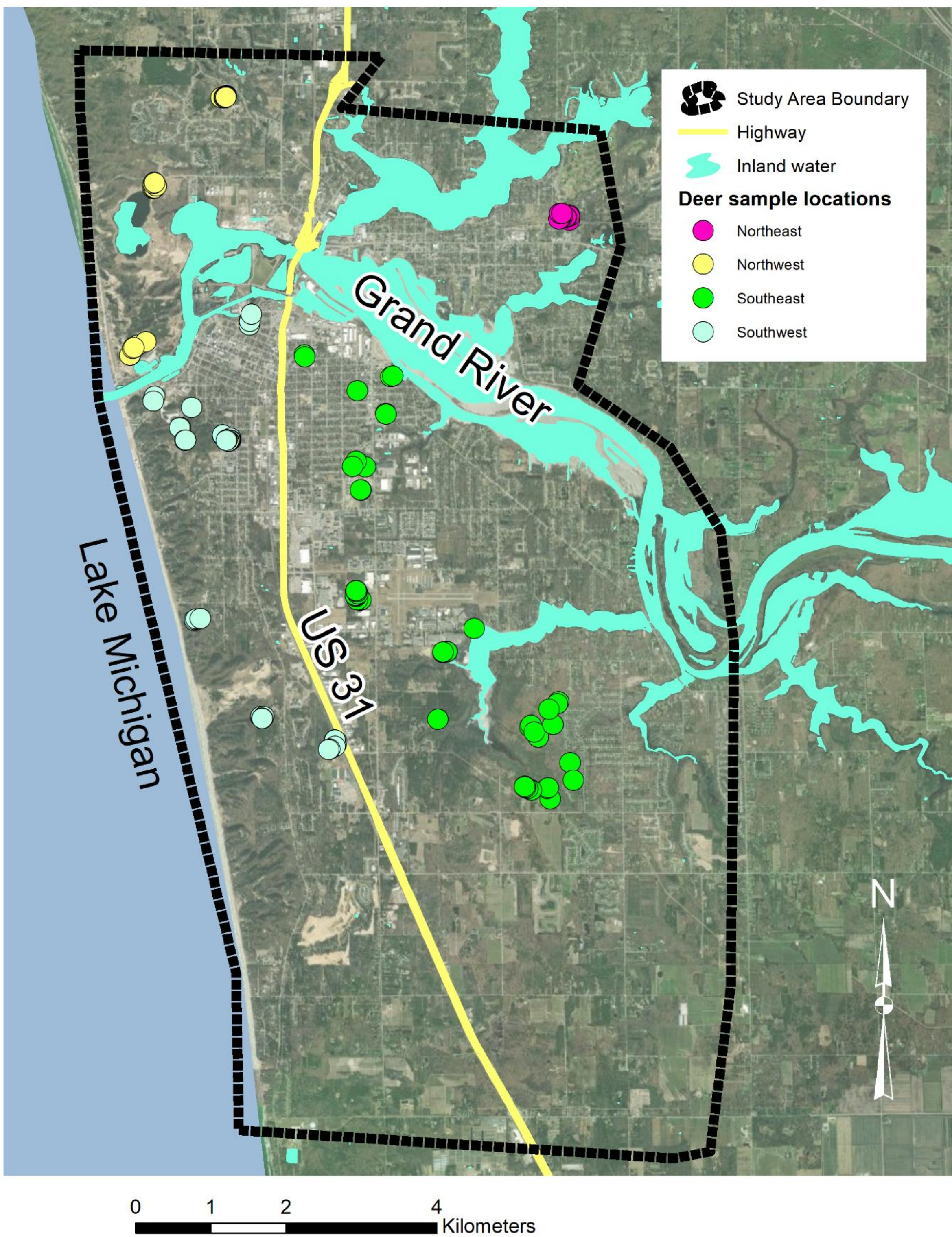

Figure 2

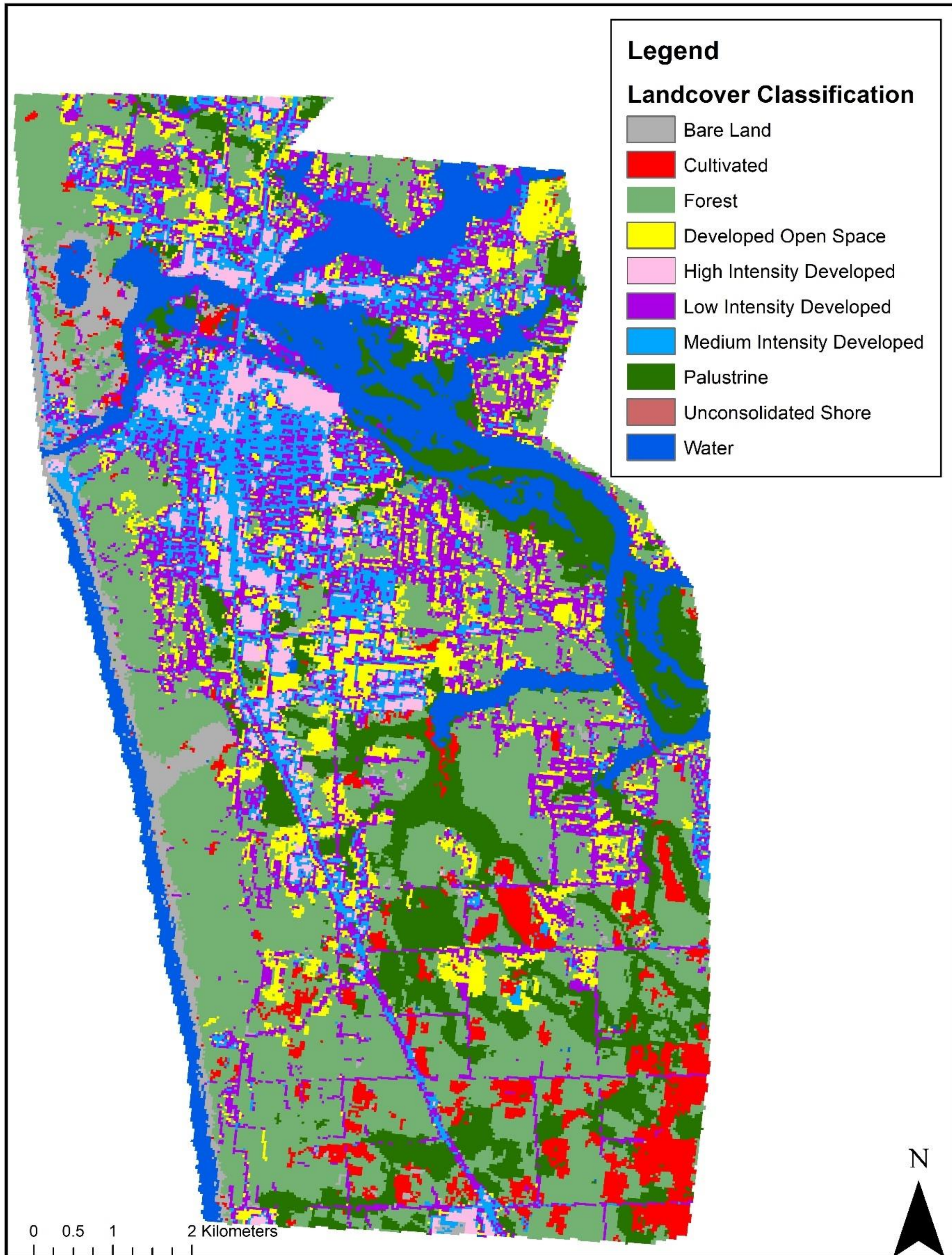

Figure 3

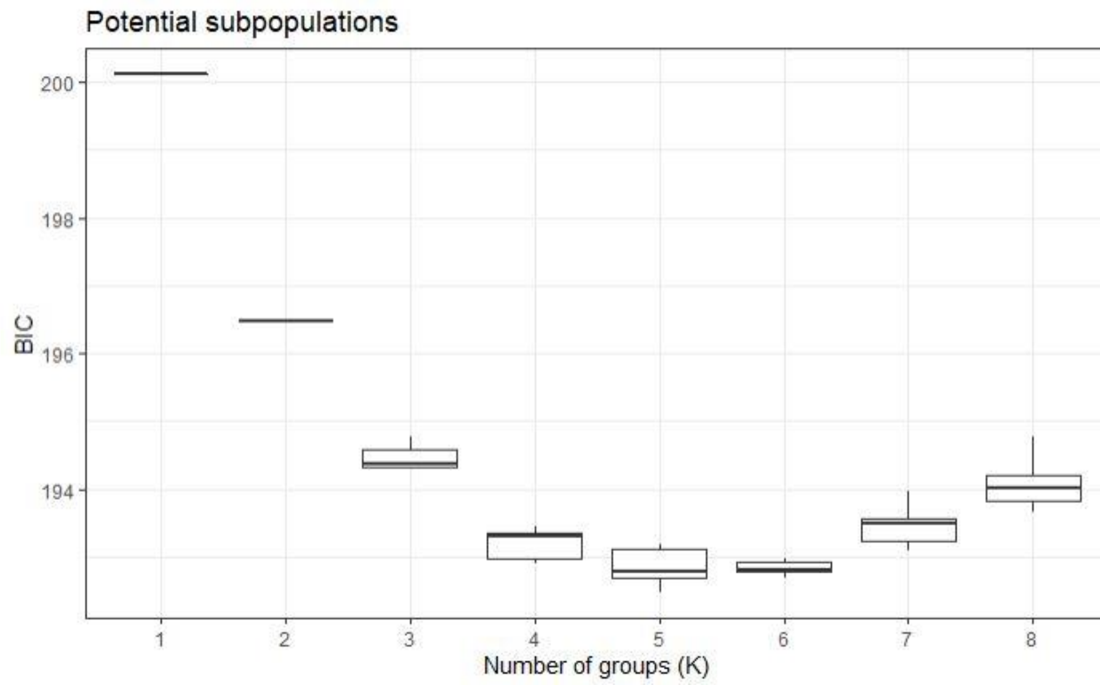

Figure 4

Figure 5

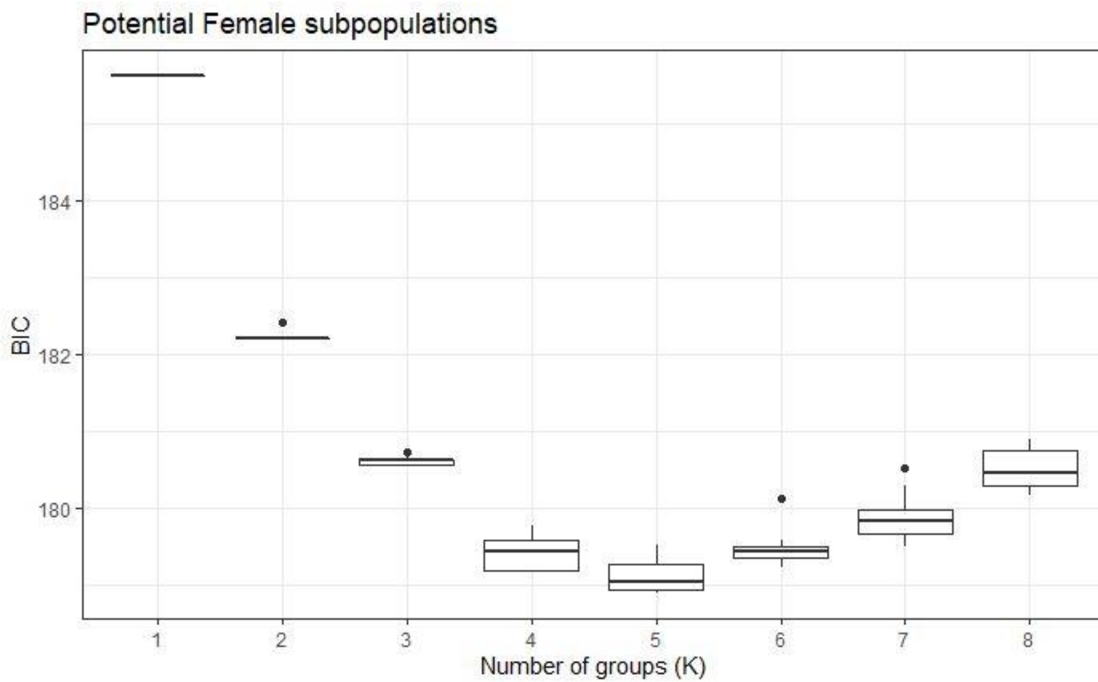

Figure 6

Figure 7

CHAPTER 3. EXTENDED REVIEW OF LITERATURE AND METHODOLOGY

Extended Review of Literature

White-tailed deer (*Odocoileus virginianus*) are a charismatic megafauna species in the United States and have economic and ecological value. Conservation in Michigan is funded through an excise tax on hunting and fishing gear and through the sale of licenses. In Michigan, hunters have the ability hunt a variety of small mammals, waterfowl, upland birds, and megafauna which include bears, elk, and deer. White-tailed deer hunting is popular among big game hunters and brings in the most money compared to the other types of hunting (Halls 1978). Funding conservation is important; however, hunters also stimulate economic growth in the communities where they hunt (Heberlein 1991).

Deer have negative and positive environmental impacts in the systems where they live. The primary driver of white-tailed deer environmental impacts is browsing. Deer contribute to the amount of regeneration in forest stands by browsing. (Kittredge and Ashton 1995). When deer browse an area intermediately, the amount of regeneration is not affected as much as over-browsing. Kittredge and Ashton (1995) noted that in forest stands where deer over browse the amount of seedling recruitment is significantly reduced. Over-browsing is potentially detrimental in habitats that are at risk, including the Eastern Hemlock where deer act as vectors for the spread of hemlock woolly adelgid that can destroy a hemlock stand (Deal 2007).

Wildlife species select habitats based on a variety of life-requisites. Life-requisites are the necessary biotic and abiotic components of a habitat in which the organism depends on. These characteristics can be modeled using a habitat suitability index (Bender and Haufler 1995). According to Bender and Haufler (1995) the major life-requisites can be separated into four

models. These models include fall and winter food, spring foods, security cover (hiding cover) and thermal cover.

The diets of deer change depending on the time of year and food availability. For example, in the spring deer will browse on new growth and herbaceous vegetation which accounts for 90% of the deer's diet (Rogers et al. 1981). As summer becomes fall there is less new growth from trees and leaves begin to fall. This means that there is a decrease in the amount of leaf and forb consumption by white-tailed deer. The decreased amount of browse from trees and forbs means that deer receive nutrients from other plants such as grasses and hard mast (Blouch 1984). Hard mast includes acorns and beechnuts, which are high in protein that allows deer to prepare for the lack of readily available food in winter. In Michigan there is a variation of snowfall across the state. In areas of high snowfall, the food sources for deer become limited. Deer must adapt to the food sources available in the winter, in most cases this means woody browse (Bender and Haufler 1995). A prolonged diet of woody browse can lead to malnourishment and starvation if not properly supplemented (Bender and Haufler 1995).

While deer rely on different food sources throughout the year they also depend on different types of cover. Deer require cover from predators and the environment (Coopperrider et al. 1986), these cover types are security and thermal cover respectively. When threatened deer run and hide, for this to be accomplished there needs to be adequate horizontal cover where deer can conceal themselves (Bender and Haufler 1995). Thermal cover is used by deer in the winter where there is cold temperatures and lack of nutrient food sources. Thermal cover reduces the heat lost by the animal and these stands are typically classified as areas with high basal area, size of the stand, and softwood crown closure (Weber et al. 1983).

Urban settings meet the requirements for habitat selection by white-tailed deer in smaller settings. For example, cities often utilize green spaces for public recreation, green spaces provide white-tailed deer sanctuaries within urban environments. Grund et al. (2002) noted that there is a greater interspersion of fragmented habitats within urban settings and deer have adapted to this change by reducing the size of their home-ranges within the urban landscape. Grund et al. (2002) also noticed that deer will use man-made infrastructure as corridors between the habitat fragments.

Genetic diversity is important for the propagation of a healthy deer herd. Deer form matrilineal groups which means that the females of a family stay in the same geographic location (Kie and Bowyer 1999). Males will disperse throughout the landscape and come back during mating season. For genetic diversity to occur male white-tailed deer must return to areas where their potential mates are genetically different.

Female deer reach reproductive maturity at the yearling stage but have a lower level of reproductive success compared to does that are 2.5 years or older. DelGiudice et al. (2007) noted that there is a lack of reproductive senesce up to 15.5 years old with a fawn rate of 1.98 fetuses per doe. White-tailed deer females are at their reproductive prime between the ages of 3 and 7 where the average fetuses per doe is almost 2 for the whole range (DelGiudice et al. 2007). The reproductive system of a white-tailed deer female contains a forked uterus. The forked uterus allows females with larger bodies who have proper nutrition to have a higher rate of multiple offspring. These twins are dizygotic meaning they come from different eggs. What is unique about deer is that the twins, while they have the same mother, can have different fathers.

Genetics influence the size of the deer as well as secondary sexual characteristics such as antler production. Antler production is influenced by a mix of genetic variability and

environmental factors (Schribner et al. 1989). During the rut, white-tailed deer will engage in physical combat for mates. It is typical that the largest male with the best antler formation has an advantage in combat. These males have a higher level of fecundity and pass on their genes (Kruuk et al. 2002).

There are multiple ways of determining if a deer is a male or female. The first of which is to physically capture the deer and look at the primary sexual organs. The second way would be through observation of the secondary sexual characteristics (i.e. antlers or no antlers). When using the observation method, it is important to note the time of year since male white-tailed deer shed their antlers in the winter and there are cases where female deer will develop antlers. Male fawns, also known as button bucks, have the potential to be classified incorrectly do to the lack of antlers at an early age. The final way that you can determine the sex of a deer is through genetic methods.

For mammals the presence of the Y chromosome leads to the development of the male sexual organs. Most mammals, marsupials and placentals, contain the SRY gene on the Y chromosome (Wallis et al. 2008). DNA needs to be extracted from individuals or collected through noninvasive methods to determine sex through the amplification of the SRY gene during polymerase chain reaction. Sex can be determined in the field if extracted directly from individuals. When using noninvasive methods sex must be determined using polymerase chain reactions (PCR). If the DNA contains the SRY gene a band will show up when running the products from the PCR on a gel during gel electrophoresis. Researchers can also use the zinc-finger intron to determine if a sample contains male or female DNA. Where the zinc-finger intron differs from the SRY gene is that there are products for both males and females. This approach targets a homologous regions of the X and Y chromosomes. After amplification, the

product for the homogametic females will consist of one band, while the heterogametic males will have two bands when using gel electrophoresis (Shaw et al. 2003).

The genetic makeup of wildlife and humans is unique unless an individual is a monozygotic twin. It is possible to distinguish between individuals using different types of genetic markers. There are four main types of genetic methods that can be used to identify individuals. These types are morphological markers (such as shape, size, coloration), protein markers, DNA based on hybridizations, and DNA markers based on hybridization. The four types of genetic methods have their own genetic markers that can be used. Each type of marker has its own set of benefits and drawbacks. For our study we used DNA markers based on PCR since our samples were likely to have low yields of DNA. This method utilizes Variable Number of Tandem Repeats which includes simple sequence repeats (SSR) also known as microsatellites (O'Brien et al. 2009, Aitken et al. 2004, Srivastava et al. 2019).

Our study utilizes microsatellites or SSRs as our primary genetic marker. A microsatellite is a portion of the DNA that is repetitive and is unique for individuals. Microsatellites can be used to identify individuals in a population (Neuman et al. 2016). Microsatellites have a varying degree of the number of alleles at their loci. When choosing microsatellites, it is important to choose highly polymorphic microsatellites which means that there is a higher number of alleles at each locus (Crawford et al. 2018)..

Microsatellites can be used to identify the number of individuals in a population. When used with maternal haplotypes researchers can create familial groups when looking at similarities in the microsatellites and mitochondrial DNA (Crawford et al. 2018).

There are multiple ways to estimate population size of deer herds. When using fecal analysis, a mark-recapture model can be used when looking at unique loci (Kohn et al. 1999).

When using genetic markers in a mark-recapture study it is assumed that your marks are not lost during the study. During a genetic mark recapture study a mark can be lost if an individual leaves the study area or if an individual dies. A common formula for mark-recapture is the Lincoln-Petersen method. This method estimates population size by multiplying the initially marked individuals and the total in the second sample then dividing by the recaptured individuals. The number of recaptures influences the size of the population. If there is a low number of recaptures, then the population estimate will be larger compared to a larger number of recaptures.

White-tailed deer thrive in urban environments because their suitable food and shelter requirements are met within green spaces and residential yards (Grund et al. 2002). A highly productive acre of land can hold two to three white-tailed deer. Deer have different social structures throughout the year. During the winter, spring and summer the male deer disperse, and the females form matrilineal groups (Kie and Bowyer 1999). Matrilineal groups remain independent of each other throughout the year. The lack of overlap of matrilineal groups has the potential to reduce the effects of overabundance (Kilpatrick and Spohr 2000).

Urban areas tend to have a higher density of white-tailed deer compared to rural areas. Increased population density and overabundance can have detrimental environmental and social ramifications. Habitats that white-tailed deer rely on for food sources can become over-browsed when the deer are at high densities. An example of an at-risk habitat type is the Eastern hemlock; the hemlock is used as a food source as well as thermal cover (Bender and Haufler 1995). Negative human-wildlife interactions occur more frequently in areas of high population densities (Porter et al. 2004). These negative interactions include vehicle collisions, destruction of gardens and wildlife entrapment on people’s property (Decker et al. 2010). Deer also are vectors for three

major diseases, and while there has not been transmission to humans, there is spread from deer to deer (Blanchong et al. 2007).

A growing concern in Michigan is the spread of chronic wasting disease, bovine tuberculosis and epizootic hemorrhagic disease (Blanchong et al. 2007). These diseases are spread through contact between deer and from the environment. The concern is that these diseases will eventually spread to humans. Understanding how our green spaces are used by wildlife can help managers reduce the negative interactions and disease while facilitating positive interactions. This understanding will increase the quality of life of the residents of urban areas and the wildlife that inhabit the cities.

Grand Haven is located in west Michigan along the shore of Lake Michigan. There is a unique relationship between the urban landscape and the deer population in the city. Movements of the deer are restricted to the west of the city by Lake Michigan and potentially to the north and east by the Grand River. The movements of deer may be influenced by the wooded areas acting as corridors for dispersal.

The city is comprised of residential, commercial and public areas with US-31 as the east-west divider of the city. The cities green spaces are comprised of beech-maple forest, oak stands, pine forests, and typical city parks. The forested areas in and around Grand Haven are also composed of Eastern hemlock. The Eastern hemlock is an important food source for deer and is also used as thermal cover (Blouch 1984). However, the hemlocks are at risk due to the spread of hemlock woolly adelgid throughout the cities. The urban deer population in Grand Haven are a potential vector for spreading the adelgid which puts these trees at risk (Deal 2007).

Within the City of Grand Haven managers do not know how many deer there are, and they do not understand how they are structured within the city. While research has been

conducted in Ingham County, Michigan, Grand Haven provides a unique set of challenges (Crawford et al. 2018). There are similarities to the study conducted in Ingham County and Grand Haven; in Meridian township where Crawford et al. (2018) conducted research the township is split by Grand River Road and in Grand Haven the city is split by US-31.

Long et al. (2010) discussed the importance of semipermeable barriers in respect to deer dispersal which influences population structuring. In Grand Haven, the main landscape features can influence dispersal are roadways, rivers, and forest. There is a gap in knowledge in knowing how deer disperse depending on their age and sex (Long et al. 2010).

Managing the deer herd is crucial for maintaining a healthy ecosystem and a suitable social carrying capacity. A social carrying capacity is the number of a species that a community or individual is willing to tolerate before management can take place (Rudolph et al. 2006). When white-tailed deer hit the ecological carrying capacity there are four major ways to control their population: Civilian hunting, Parks sharpshooting, Police shooting, and Conservation office/government agency removal (Doerr et al. 2001). Doerr et al. (2001) stresses that the best removal method in terms of cost benefit analysis is the use of hunters to remove deer. When hunters remove deer populations the city makes money from the application fees and the tags that are sold. Management plans should consider the safety of the public before being implemented. The residents should also be involved in the creation of the management plan so the social carrying capacity can be considered and the public can be informed about the number of deer being removed from the city.

Extended Review of Methodology

Sample Collection and Processing

The sample area was stratified into four sections by prominent landscape features including US-31 and the Grand River. White-tailed deer fecal samples were then collected from public green spaces between July 2019 to March 2020. Public green spaces were determined by using property mapping through Ottawa County GIS services (gismiottawa.org). We collected fecal samples in a two-hour time frame at each sampling location. Once fecal piles were identified, we collected 8-10 pellets were collected we stored them in 50 ml falcon conical tube. Each sample was labeled with a 3-digit numerical code ending with a letter that corresponded with the sampling day (i.e. 002A). Fecal samples were only collected if they were fully intact, and not dried or, covered in sand. Global positioning system coordinates were taken at each fecal sample location for future spatial analysis. We swabbed fresh samples in the field using a histobrush dipped in cell lysis buffer. After swabbing the samples to remove the mucosal membrane and DNA, we left the brush in the conicals for transport back to the lab for further processing. The samples and the lysis buffer were kept on ice throughout the transportation process to prevent degradation.

We collected tissue samples from both male and female hunter-harvested white-tailed deer heads acquired from a local deer processor. We dissected the heads and removed tissue from the masseter muscle and removed the lymph nodes from the throat. Upon collection, the samples were put on ice and transported to the -80°C freezers in the lab. Once the samples were completely frozen, we pulverized them into a fine dust using a mortar and pestle. To keep the samples frozen during the pulverization process we kept the mortar on dry ice while pouring in liquid nitrogen. The powder was collected in a 50 ml conical and stored at -80°C until it was

processed. Using the protocol in Baise et al. (2002) we placed the powder in a 2 ml Eppendorf tube and added 800 µl of an extraction solution (50 mM Tris-HCL, 25 mM EDTA, 400 mM NaCl), 100 µl 10% SDS, and 20 µl Proteinase K to the powder. The Eppendorf tube with powder and solution was the incubated at 65°C for three hours. At the end of the three-hour incubation period 300 µl of NaCl were added to precipitate the proteins. After the precipitation of the proteins the Eppendorf tube was kept at 4°C for 15 minutes. The sample was centrifuged for 20 minutes at 25,000 x g after the cold storage. Next, we transferred 500 µl of the supernatant to another Eppendorf tube and added 500 µl of 8 M guanidine hydrochloride and 0.49 M ammonium acetate solution. The sample was kept in mild agitation for 90 minutes, after which we precipitated the nucleic acids with 800 µl of cold 100% isopropyl alcohol and then centrifuged for five minutes at 8000 x g. We washed the pellets with 400 µl of 70% isopropyl alcohol after 5 minutes. We stored the dry pellets at 4°C after resuspending them in 150 µl TE buffer (10 nM Tris-HCL, 1mM EDTA, 50 ug/µl RNAse). The positive control DNA was diluted to a 1:10 ratio to use in reactions.

Once the DNA was swabbed from the fecal collections, we eluted the DNA following the protocol published by Zymo Research. We suspended the histobrush in a solution that contained 20 µl of proteinase K (50 µl of 10mM Tris HCL, 5 µl 1 nM $CaCl_2$, 2.5 ml 100% Glycerol, 2.445 ml Nuclease Free Water), 200 µl of elution buffer and 200 µl of a cell buffer in a microcentrifuge tube. We placed the microcentrifuge tube in a 55°C water bath to incubate for 10 minutes. We removed the brush and added one volume of genomic binding buffer and mixed. We added the sample to a spin column and ran the sample in the centrifuge for one minute at 12,000 x g. We added 400 µl of DNA prewash and repeated the centrifuge process keeping the speed and time the same. Once the DNA prewash was discarded, we centrifuged two more times using 700 µl

and 200 µl g-DNA wash buffer, respectively. Finally, we eluted the DNA by adding 30 µl of elution buffer, incubating the sample at 55°C for 5 minutes and then centrifuging. Once the first spin was done, we added 30 µl more of elution buffer and centrifuged the sample one last time giving us 60 µl of eluted DNA. We then diluted the DNA to a 1:10 ratio for the sequential tests.

To determine the sex from the fecal samples we use Polymerase Chain Reactions of the zinc finger intron as well as SRY. The zinc finger intron has base pair variation between the X and Y locus which can be determined by running samples across 2% Sodium Borate (SB) (2 grams SB agarose, 100 ml SB buffer) agarose gel during gel electrophoresis using an SB running buffer (100 ml 50x SB stock, 4.9 liters Deionized water) made from 50x SB stock (20 g NaOH, 120g H_3BO_3, Bring up to 1 liter of Deionized H_2O). The lanes were loaded with 15 µl of the PCR contents (12.5 µl Master Mix 2x, 0.5 µl Forward Primer 50 uM, 0.5 µl Reverse Primer 50 uM, 10 µl DNA, 1.5 µl nuclease free water) and 5 µl of gel green and then the gels were run at 270 volts. When viewed on an ultraviolet transilluminator, a male sample contained two bands with the zinc finger intron while female samples contained one. Given the biology of white-tailed deer, males are less likely to congregate; thus, if there was an excess of male positive samples from the same sampling location, we would run SRY to verify if the male samples were a false positive or not.

For this study we chose 10 microsatellites that had been used in published peer review deer research papers such as Crawford et al (2018) and Anderson el (2002). When choosing our microsatellites, we were focused on how polymorphic the loci are. For example, obcam, has 17 alleles and a dinucleotide repeat making the microsatellite highly polymorphic. To verify our annealing temperatures, we used a gradient on the Eppendorf Mastercycler Nexus thermocycler referencing published annealing temperatures in Anderson et al. (2002) and Crawford et al.

(2018). During our testing we used 50 uM forward and reverse microsatellite primers in our reaction. We used the male control DNA that we harvested from the deer heads as our positive control and nuclease free water as our negative control. When temperatures were lower than optimal, there was a higher level of non-specificity displayed on the gels and were ruled out. To determine whether there was non-specificity or if there was product after PCR we ran the products on a 2% Sodium Borate (SB) agarose gel using gel electrophoresis.

After we determined the optimal temperatures for PCR, we ran one microsatellite per plate in the thermocycler using the 250 uM fluorescent primers. The microsatellites were then split into two separate groups for fragment analysis. Our PCR master mix included 12.5 µl Master Mix 2x, 0.5 µl Forward Primer 250 uM, 0.5 µl Reverse Primer 250 uM, 10 µl DNA, 1.5 µl nuclease free water, and the steps for our PCR process were as follows: Initialization for 10 minutes at 95 °C, denaturation for 30 seconds at 95 °C, annealing at the specific temperatures (i.e., Table 1) for 30 seconds, elongation at 72 °C for 1 minute, steps 2–4 were repeated 38 times, then lastly there was a final elongation at 72 °C for 5 minutes. We used the male muscle DNA as our positive control and nuclease free water as our negative control. We determined the groups based on the fluorescent tag the primers had and the number of base pairs. We created two final plates containing 5 microsatellites each using 10 µl of each microsatellite PCR product (Table 1).

To determine the identity of individuals, we used CERVUS 3.0.7 (Field Genetics, London, United Kingdom). For identity analysis CERVUS compares the genotype of one sample to that of all others. If there was a direct match, then it was recorded as a recapture. While sampling in the same day, if we had an individual's feces picked up twice this was not considered a recapture because it could not be considered independent events. CERVUS also used fuzzy matching which allows researchers to set the desired number of matching genotypes

for positive recaptures for their population estimate. Individuals needed to match 9 of the microsatellites on separate days to be determined recaptures. We separated the samples into 2 time periods, summer (July through September 2019) and fall/winter (October through March 2020). The number of repeat captures during the second time period were then used in the Lincoln-Peterson mark-recapture model to determine a population estimate. We used the equation:

$$\widehat{N} = \frac{MC}{R}$$

where,

$\widehat{N}$ = population size estimate

M = total number of marked individuals during the first time period

C = total number of individuals captured during the second time period

R = number of recaptured individuals during the second time period

We also computed the standard deviation ($\hat{S}$) and 95% confidence limit ($N \pm 1.96 * \hat{S}$) using the equation:

$$\hat{S} = \sqrt{\frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(R+2)}}$$

Lincoln-Peterson mark-recapture model has five assumptions (Seber 1982): 1) the population is closed, 2) the marks cannot be lost or missed. 3) marked individuals mix randomly with the unmarked individuals, 4) there is an equal opportunity of capture, and 5) the mark does not change the behavior or increase the risk of mortality for the individual. The overall inbreeding coefficient (F_{IS}) and estimates of Hardy Weinberg Equilibrium were calculated in GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). To determine the number of subpopulations

within our study site we used discriminate analysis of principal components (DAPC) (Jombart et al. 2010). We also ran the DAPC analysis using only the females from 148-samples (i.e., that amplified at a minimum of 6 microsatellites) to determine if females would have different clustering patterns without the males considering they stay in matriarchal groups. To determine the number of populations we used the lowest Bayesian information criterion (BIC). When running the DAPC we were originally testing to see if the value for k was in the 10–12 range. We eventually narrowed the range down from 3–8 and determine that the number of genetic clusters was 5 for each subset within the study. We visualized the membership of individuals in each group using an assignment plot.

Table 1: The ten microsatellites were split into two groups based on the number of base pairs and the fluorescent tag for the 250 mM primer. The microsatellites were pooled into two well plates corresponding with their group for fragment analysis.

Group 1		
Microsatellite	Fluorescent Tag	Base pairs
Cervid	FAM	288-336
N	FAM	96-124
OarFCB193	NED	106-151
IGF1	FAM	106-151
Q	PET	233-281

Group 2		
Microsatellite	Florescent Tag	Base pairs
RT9	PET	101-127
BM4107	VIC	157-181
RT7	VIC	207-243
BL42	FAM	233-266
Obcam	PET	184-220

APPENDICES

Table A1: Locations of the 148 fecal samples used for this study.

Sample	Latitude	Longitude
6D	43.05827	-86.2413
7D	43.03044	-86.18897
10D	43.05901	-86.2082
11D	43.05055	-86.2084
12D	43.04713	-86.2076
13D	43.04711	-86.2076
14D	43.04993	-86.209
18D	43.05784	-86.2415
21D	43.05827	-86.2413
23D	43.04715	-86.2077
25D	43.05772	-86.2415
9E	43.02766	-86.1942
10E	43.02779	-86.1935
11E	43.02779	-86.1935
12E	43.02773	-86.1942
13E	43.06318	-86.2453
15E	43.06408	-86.2448
16E	43.02724	-86.19485
17E	43.06436	-86.24415
19E	43.03059	-86.18913
20E	43.02777	-86.1942
3g	43.05359	-86.2302
4g	43.05367	-86.2302
5g	43.05329	-86.2289
6g	43.05305	-86.2289
8g	43.05306	-86.2289
12g	43.05294	-86.2291
14g	43.05304	-86.229
15g	43.05295	-86.2292
16g	43.05296	-86.2292
17g	43.05293	-86.2292
18g	43.05289	-86.2293
22g	43.05306	-86.2295
23g	43.05312	-86.2295
24g	43.05303	-86.2296
001i	43.05631	-86.2036
002i	43.05621	-86.2036
003i	43.06684	-86.2259
007i	43.06735	-86.2259
010i	43.06728	-86.2258

Sample	Latitude	Longitude
012i	43.0681	-86.2256
019i	43.05457	-86.2371
021i	43.05451	-86.2372
008J	43.01994	-86.2239
010J	43.02001	-86.2239
016J	43.02001	-86.2234
018J	43.01974	-86.2236
020J	43.0198	-86.2237
021J	43.01977	-86.2236
022J	43.01976	-86.2236
001K	43.06328	-86.2169
002K	43.06303	-86.2169
005k	43.03408	-86.2078
007k	43.03394	-86.2077
008k	43.03398	-86.2076
009k	43.03398	-86.2082
010k	43.03434	-86.2084
011k	43.03444	-86.2084
012k	43.03457	-86.2084
013k	43.03451	-86.2085
014k	43.03469	-86.2081
015k	43.03492	-86.2082
016k	43.03509	-86.2083
017k	43.0351	-86.2084
018k	43.0351	-86.2084
001L	43.06318	-86.2454
005L	43.06399	-86.2449
008L	43.06409	-86.2447
010L	43.08316	-86.2417
011L	43.0832	-86.2415
013L	43.08319	-86.2414
015L	43.08344	-86.2412
016L	43.08344	-86.2412
017L	43.08372	-86.2416
018L	43.08385	-86.2415
019L	43.08383	-86.2414
002M	43.01021	-86.1767
004M	43.01146	-86.1772
006M	43.01154	-86.177
008M	43.01127	-86.1798
009M	43.01124	-86.1797
011M	43.01149	-86.1804
012M	43.01142	-86.1803

Sample	Latitude	Longitude
014M	43.01159	-86.1807
015M	43.01155	-86.1807
017M	43.0117	-86.1808
018M	43.01166	-86.1808
019M	43.01165	-86.1808
002N	43.01674	-86.2116
003N	43.01675	-86.2116
005N	43.01702	-86.2115
008N	43.01705	-86.2119
009N	43.0172	-86.2118
011N	43.0172	-86.2117
012N	43.01645	-86.2117
013N	43.01608	-86.2126
014N	43.01604	-86.2128
022N	43.053	-86.2363
023N	43.05302	-86.2364
025N	43.05304	-86.2363
001o	43.01453	-86.1734
002o	43.01884	-86.18
003o	43.01759	-86.1786
004o	43.019	-86.1798
006o	43.0191	-86.1762
007o	43.0182	-86.1793
008o	43.01244	-86.1729
009o	43.02185	-86.1753
010o	43.0191	-86.1762
011o	43.0216	-86.1755
012o	43.0209	-86.1769
001p	43.07946	-86.1737
002p	43.08009	-86.1737
004p	43.07993	-86.1737
005p	43.07979	-86.1742
007p	43.07984	-86.1743
009p	43.07979	-86.1749
010p	43.07991	-86.1751
011p	43.07983	-86.1751
013p	43.07971	-86.1753
014p	43.0797	-86.1753
015p	43.07965	-86.1754
017p	43.0796	-86.1754
018p	43.08025	-86.1753
020p	43.08025	-86.1749
021p	43.08028	-86.1749

Sample	Latitude	Longitude
022p	43.08025	-86.175
005q	43.09426	-86.2298
008q	43.09423	-86.2298
016q	43.09422	-86.2299
023q	43.09417	-86.23
024q	43.09414	-86.2299
029q	43.09417	-86.2301
039q	43.09404	-86.2306
045q	43.09409	-86.23
046q	43.094	-86.2301
047q	43.09409	-86.23
048q	43.09412	-86.2299
049q	43.09413	-86.2298
050q	43.0941	-86.2298
024r	43.05695	-86.2353
030r	43.03143	-86.2347
031r	43.03157	-86.2347
033r	43.03166	-86.2345
048r	43.03171	-86.2337
005s	43.0607	-86.2028
010s	43.06078	-86.2025
021s	43.01972	-86.195

Table A2: Diploid format for the 148 samples in the subset used in the DAPC and the mark recapture.

Sample	Pop	Cervid	N	RT7	RT9	Q	Obcam	Oar	IGF1	BM4107	BL42
001p	NE	160178	304304	221221	101101	277277	222222	110116	124124	139151	243243
002p	NE	160182	300320	221221	115115	249257	222222	110116	124124	139147	239239
004p	NE	160182	300300	221221	109109	265273	222222	116116	124124	139139	243243
005p	NE	182182	300320	217237	105113	265273	212222	100114	124124	151157	233243
007p	NE	160178	296296	225225	105123	000000	222222	110110	124124	147147	243243
009p	NE	160160	312320	221221	113117	241273	222222	110116	124124	139151	239249
010p	NE	160184	300320	221229	117117	241253	208222	114116	124124	139147	235235
011p	NE	160184	300320	221221	117117	000000	204208	116116	124124	139147	239243
013p	NE	178186	300300	221221	117117	249249	222222	116116	114124	147147	233265
014p	NE	160178	300320	225233	113113	253273	208222	100110	124124	151151	233235
015p	NE	184184	300320	221221	113117	257257	222222	94116	124124	139151	233233
017p	NE	178184	320320	219219	117123	000000	222222	110116	124124	139151	235241
018p	NE	160178	312320	221241	113117	273273	208222	110116	124124	139151	243243
020p	NE	182182	300320	221221	113117	253273	222222	98116	124124	139151	239243
021p	NE	178184	300320	000000	117123	241253	208208	110116	124124	139151	243243
022p	NE	160178	312320	229229	113117	253273	204208	110116	124124	139151	243243
13E	NW	160178	312320	229229	113117	253273	204208	110116	124124	139151	243243
16E	NW	172182	296296	000000	115115	265273	206212	106114	124124	157157	241243
001L	NW	178178	000000	229229	127127	241273	202202	9494	124124	147147	235235
005L	NW	172196	300308	000000	117117	241257	222222	98114	138138	157157	239239
008L	NW	184196	304312	202202	117117	233233	000000	000000	150150	173177	233239
010L	NW	178186	296308	217217	105121	241269	218218	106110	118118	139161	235249
011L	NW	178186	308308	217217	113127	253273	000000	98114	118118	139147	235239
013L	NW	160160	300312	217217	113117	269273	206222	98116	124124	143161	235239
015L	NW	178186	296296	225225	105117	253273	208222	98114	124124	139151	235239
016L	NW	160182	300304	221221	117121	265265	196204	98116	124124	143191	233233
017L	NW	170170	308308	237237	105105	233233	236236	116120	108108	147173	233239
018L	NW	160178	300300	209217	117117	233273	204206	114116	124124	137151	233235
019L	NW	160172	304316	217221	117123	249249	202218	106116	124124	157161	235235

Sample	Pop	Cervid	N	RT7	RT9	Q	Obcam	Oar	IGF1	BM4107	BL42
005q	NW	178178	304308	209219	115115	277277	184184	98114	124124	000000	249249
008q	NW	178178	304308	000000	105105	241273	000000	98114	124124	137137	243243
016q	NW	178178	312320	000000	101109	233233	184196	9898	124124	000000	249249
023q	NW	178196	300316	202202	109115	249277	218218	106106	124124	157157	235241
024q	NW	160172	304308	213213	127127	249249	204212	9898	124124	139157	000000
029q	NW	178178	304304	213213	113115	249257	218222	9494	124124	139143	239261
039q	NW	172172	328328	219229	000000	233241	184222	000000	124124	157157	235241
045q	NW	182182	304312	203203	123123	233253	222222	9494	124124	139139	235241
046q	NW	178182	336336	219233	117117	233257	222222	116116	124130	139139	235235
047q	NW	170184	308312	219225	113117	253273	218218	110114	124130	143157	233239
048q	NW	182182	308308	229229	117117	257273	222222	120120	124124	139139	235239
049q	NW	182186	312312	209219	117117	253253	218222	106116	124138	139139	235239
050q	NW	178178	320320	213219	115115	253253	222222	100110	124124	139139	235249
10D	SE	160178	312312	219225	105113	249269	222222	110114	124124	137143	233233
11D	SE	160170	308308	209221	117123	269273	202206	98106	130138	143151	235239
12D	SE	182182	308312	209225	109117	241249	204222	98110	124138	139137	235235
13D	SE	182186	308320	225225	113115	233253	208222	100114	124124	139151	235239
14D	SE	182186	296312	219219	113117	249277	222222	106114	124124	137143	241241
23D	SE	184184	308308	225237	115115	233253	222222	114114	124124	137137	235235
9E	SE	160186	304336	213225	109121	265277	202206	100116	124124	143161	241249
10E	SE	160172	328328	219225	105121	233233	212212	110116	124124	139157	239239
11E	SE	186196	296296	219225	105121	249265	196222	98116	124124	143157	235241
12E	SE	172182	308308	229229	113123	233257	206222	110114	124124	143151	235265
15E	SE	172178	300308	229229	101113	233257	184184	114114	124124	143151	235239
17E	SE	172182	308308	000000	000000	233233	184202	114114	124124	000000	000000
19E	SE	172182	308308	219219	101101	233257	184204	110114	124124	143143	235241
20E	SE	178182	304304	219219	121123	257273	196222	9898	124124	143143	241241
001i	SE	160182	296296	241241	101121	269273	190190	106110	124124	143161	235241
001K	SE	160182	300300	225225	109117	257269	206208	98106	124130	139139	239239
002K	SE	160160	320320	219225	113117	241265	208208	100106	124124	139139	239243
005k	SE	182186	308324	225225	105117	253269	206212	106114	124124	143157	235235

Sample	Pop	Cervid	N	RT7	RT9	Q	Obcam	Oar	IGF1	BM4107	BL42
007k	SE	184196	300328	221221	105105	000000	206206	114116	124124	143157	235243
008k	SE	182196	300308	221221	105117	253277	206206	114114	124130	157157	235235
009k	SE	178178	300308	221221	105117	233233	222222	110114	124124	157157	235243
010k	SE	172182	308336	221221	113117	249269	196222	110116	124124	139161	233241
011k	SE	160184	300316	219233	117117	265273	184196	100110	124124	139161	239243
012k	SE	184196	300308	203221	105109	277277	196206	110116	124130	143157	235261
013k	SE	182182	312336	221221	113117	249269	204222	110116	124124	139161	233241
014k	SE	160160	300316	219219	117117	265273	222222	100110	108124	139161	239261
015k	SE	184184	308328	203209	105117	253257	190212	94106	130138	157157	235235
016k	SE	182182	308308	203213	117117	000000	206222	106114	124130	139139	239239
017k	SE	182182	308336	203221	109117	269269	204218	110116	124124	139161	233241
018k	SE	170170	308308	237237	105105	233233	236236	116120	108108	147173	233239
002M	SE	170182	300300	221237	109123	249257	206206	110114	118118	143161	239239
004M	SE	170170	300308	219225	113123	249257	000000	98114	124124	143161	235239
006M	SE	000000	316328	217217	113123	249249	000000	000000	124124	143161	233239
008M	SE	170182	336336	209221	105121	253257	202202	94116	130130	161161	235241
009M	SE	178178	304304	217221	123123	241241	222222	9494	118118	139151	235235
011M	SE	182182	304320	203203	113123	241257	204204	000000	000000	139139	235241
012M	SE	182182	296328	203203	113123	233249	204218	9494	138138	139177	233239
014M	SE	178178	308336	000000	113117	257277	222222	110116	118118	143143	235239
015M	SE	172184	000000	219219	109115	249253	202222	100100	124124	143143	233235
017M	SE	186186	000000	209219	115121	249273	208218	106114	124124	139157	239243
018M	SE	000000	304304	209213	113115	233277	204218	000000	124124	139139	233239
019M	SE	178196	308312	213217	109115	249253	222222	100100	124124	139143	239239
001o	SE	178182	304336	219221	113115	249253	218222	98116	124138	161161	235241
002o	SE	178182	320320	219225	105117	257273	206218	100116	124130	139161	239241
003o	SE	182186	320320	219225	115117	253273	204206	106116	124130	139161	235239
004o	SE	182182	304304	221229	105117	257273	222222	114116	124124	139139	235239
006o	SE	172178	304320	219225	113115	277277	222222	114114	108124	147161	235241
007o	SE	172182	304304	225229	117123	241273	206206	110114	124130	139157	235239
008o	SE	170182	300300	225237	117123	241249	206206	110114	124124	143147	239239

Sample	Pop	Cervid	N	RT7	RT9	Q	Obcam	Oar	IGF1	BM4107	BL42
009o	SE	178178	304304	213219	101115	257273	204204	100116	124124	139151	235239
010o	SE	196196	324324	219233	113117	241257	218222	106114	118138	139157	235239
011o	SE	184184	296336	225229	115117	257269	222222	114114	124138	139147	239239
012o	SE	178184	300304	219229	113117	241257	202208	110116	124130	137137	239241
005s	SE	186186	300308	213221	105105	000000	222222	9494	124124	139157	235241
010s	SE	164186	308336	213221	105105	000000	202202	000000	124124	139151	235241
021s	SE	182186	308308	213221	101115	233233	204204	9494	138138	139151	235241
6D	SW	170182	308308	219225	105109	241257	222222	98114	124124	147161	235241
7D	SE	182182	296312	219237	113117	249277	218222	106114	124124	137143	239239
18D	SW	178178	296308	225225	101121	249249	222222	116116	124138	137173	233243
21D	SW	164172	300308	209225	115115	241241	206212	106114	124138	139139	235235
25D	SW	182182	296304	219237	109117	241265	222222	98116	124138	151161	235239
3g	SW	178182	308308	221237	109117	257265	196206	98116	118118	151161	235239
4g	SW	182182	308308	221237	109117	249249	196204	100116	124124	151161	235235
5g	SW	160182	304308	219221	109121	249265	204208	98100	124124	151161	235243
6g	SW	178182	308308	221237	109117	257265	000000	100116	124124	151161	235239
8g	SW	182182	300308	217221	105109	249249	000000	98106	124124	157161	235239
12g	SW	172178	296308	202233	105115	241241	196206	100116	124124	139177	235239
14g	SW	178182	300308	221221	101117	249257	206222	100116	124150	161161	233239
15g	SW	182182	308308	225237	101117	257265	196206	100116	124124	151161	235239
16g	SW	182186	300312	221237	127127	249249	196196	98114	124124	151151	235239
17g	SW	182182	308308	203221	109113	241265	202202	100120	124124	161161	239239
18g	SW	178182	308308	225237	109117	257265	196206	100116	114124	151161	235239
022N	SW	178182	000000	219219	121121	233233	190190	9494	124124	139151	235241
22g	SW	160182	308308	221225	109121	249265	206222	100100	124124	151161	235243
23g	SW	182186	308308	221225	109113	241265	202204	100116	124150	151161	239239
24g	SW	178182	300308	219225	101109	241241	204222	100110	124124	157161	233239
003i	SW	182182	296328	209225	109115	257257	206212	110114	124124	137143	235239
007i	SW	170182	300328	209217	000000	249249	000000	106114	124124	137151	235239
010i	SW	182196	296328	203225	113117	257257	208208	9898	118120	137143	235239
012i	SW	182186	300304	219225	117117	253257	218218	114114	124124	137143	239239

Sample	Pop	Cervid	N	RT7	RT9	Q	Obcam	Oar	IGF1	BM4107	BL42
019i	SW	160182	300304	217217	000000	249249	208208	94116	124124	139139	249249
021i	SW	184184	304336	217217	117117	273273	204204	100114	124124	137151	239249
008J	SW	160182	308308	225237	117117	000000	206206	116116	124124	137151	239249
010J	SW	172182	300300	221221	121127	257269	206222	106114	124138	137147	235235
016J	SW	184186	308336	217229	105105	265265	212212	114116	124124	151157	233243
018J	SW	160170	308308	209221	117123	269273	202206	98106	130138	143151	235239
020J	SW	172182	300308	221221	117117	257269	206206	106114	124124	151151	235235
021J	SW	182182	308308	202202	117117	257257	196222	116116	108124	139139	235235
022J	SW	184186	308336	219229	109109	265265	212212	110116	124130	151157	233243
002N	SW	178178	304304	213213	117117	277277	218218	98116	124124	143151	239265
003N	SW	178178	304304	213213	113115	249257	218222	9494	124124	139143	239261
005N	SW	178178	308308	221221	117117	249257	222222	114116	124124	151161	233239
008N	SW	178178	296296	202202	121121	249249	202202	94106	108114	139161	235241
009N	SW	182186	304336	213225	115117	257269	202202	100116	118118	143143	235239
011N	SW	172182	304304	213225	115117	257269	222222	100116	118118	143143	239249
012N	SW	160178	296296	209237	121123	265265	212236	106114	108108	139151	235241
013N	SW	178196	308308	202202	000000	000000	196196	9494	118118	143157	261261
014N	SW	164178	000000	213225	115115	257257	222222	9494	120120	139139	235241
023N	SW	178178	300308	202202	105109	233241	204204	9494	150150	161176	235239
025N	SW	172182	304336	229229	113117	257277	204222	114116	124124	137143	235239
024r	SW	178178	000000	203203	000000	233273	222222	100100	124124	139139	239241
030r	SW	178178	300308	202202	105109	233241	204204	9494	150150	161176	235239
031r	SW	172182	304336	229229	113117	257277	204222	114116	124124	137143	235239
033r	SW	178178	000000	203203	000000	233273	222222	100100	124124	139139	239241
048r	SW	160164	308312	209221	109115	000000	000000	9494	124124	139143	235241

BIBLIOGRAPHY

Aronson, M. F., Lepczyk, C. A., Evans, K. L., Goddard, M. A., Lerman, S. B., MacIvor, J. S., Nilon, C.H., Vargo, T. (2017). Biodiversity in the city: Key challenges for urban green space management. Frontiers in Ecology and the Environment, 15(4), 189–196.

Bender, L. C., and J. B. Haufler. (1995). A white-tailed deer HSI for the Upper Great Lakes Region. Pages 121-151 in Roloff, G. J. Using an ecological classification system and wildlife habitat models in forest planning. Dissertation, Michigan State University, East Lansing, USA.

Blanchong, J. A., Scribner, K. T., Kravchenko, A. N., & Winterstein, S. R. (2007). TB-infected deer are more closely related than non-infected deer. Biology Letters, 3(1), 103–105.

Blouch, R. I. (1984). Northern great lakes states and Ontario forests. White-tailed deer ecology and management. Stackpole Books, Harrisburg, Pennsylvania, USA, 391-410.

Cooperider, A. Y., Boyd, R. J., & Stuart, H. R. (1986). Inventory and Monitoring of Wildlife Habitat', US Dept. Interior, Bur. Land Management, Service Center, Denver, CO.

Crawford, J. C., Quinn, A. D., Williams, D. M., Rudolph, B. A., Scribner, K. T., & Porter, W. F. (2018). Fine-scale spatial genetic structure of deer in a suburban landscape. The Journal of Wildlife Management, 82(3), 596–607.

Deal, I. (2007). Life History of Hemlock Woolly Adelgid, *Adelges tsugae* Annand, on Eastern Hemlock, *Tsuga canadensis* (L.) Carriere, in the Southern Appalachians and Assessment of Egg Releases of *Sasajiscymnus tsugae* (Sasaji and McClure) for Its Management. Masters Theses.

Decker, D. J., Evensen, D. T. N., Siemer, W. F., Leong, K. M., Riley, S. J., Wild, M. A., Castle, K.T., Higgins, C. L. (2010). Understanding Risk Perceptions to Enhance Communication about Human-Wildlife Interactions and the Impacts of Zoonotic Disease. ILAR Journal, 51(3), 255–261.

DelGiudice, G. D., M. S. Lenarz, and M. C. Powell. 2007. Age-Specific Fertility and Fecundity in Northern Free-Ranging White-Tailed Deer: Evidence for Reproductive Senescence? Journal of Mammalogy 88:427–435.

Doerr, M. L., McAninch, J. B., & Wiggers, E. P. (2001). Comparison of 4 Methods to Reduce White-Tailed Deer Abundance in an Urban Community. Wildlife Society Bulletin (1973-2006), 29(4), 1105–1113.

Grund, M. D., McAninch, J. B., & Wiggers, E. P. (2002). Seasonal Movements and Habitat Use of Female White-Tailed Deer Associated with an Urban Park. The Journal of Wildlife Management, 66(1), 123–130.

Halls, L. K. (1978). White-tailed deer. Pp. 43-65, in Big game of North America: ecology and management (J. L. Schmidt and D. L. Gilbert, eds.). Stackpole Books, Harrisburg, Pennsylvania.

Heberlein, T. A. (1991). Changing Attitudes and Funding for Wildlife: Preserving the Sport Hunter. Wildlife Society Bulletin (1973-2006), 19(4), 528–534.

Kie, J. G., & Bowyer, R. T. (1999). Sexual Segregation in White-Tailed Deer: Density-Dependent Changes in Use of Space, Habitat Selection, and Dietary Niche. Journal of Mammalogy, 80(3), 1004–1020.

Kilpatrick, H. J., & Spohr, S. M. (2000). Spatial and Temporal Use of a Suburban Landscape by Female White-Tailed Deer. *Wildlife Society Bulletin (1973-2006)*, *28*(4), 1023–1029.

Kittredge, D. B., & Ashton, P. M. S. (1995). Impact of Deer Browsing on Regeneration in Mixed Stands in Southern New England. *Northern Journal of Applied Forestry*, *12*(3), Kittredge, D. B., & Ashton, P. M. S. (1995). Impact of Deer Browsing on Regeneration in Mixed Stands in Southern New England. *Northern Journal of Applied Forestry*, *12*(3), 115–120.

Kohn, M. H., York, E. C., Kamradt, D. A., Haught, G., Sauvajot, R. M., & Wayne, R. K. (1999). Estimating population size by genotyping faeces. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1420), 657–663.

Kruuk, L. E. B., J. Slate, J. M. Pemberton, S. Brotherstone, F. Guinness, and T. Clutton-Brock. 2002. Antler Size in Red Deer: Heritability and Selection but No Evolution. Evolution 56:1683–1695.Long, E. S., Diefenbach, D. R., Wallingford, B. D., & Rosenberry, C. S. (2010). Influence of Roads, Rivers, and Mountains on Natal Dispersal of White-Tailed Deer. *The Journal of Wildlife Management*, *74*(6), 1242–1249

Neuman, T. J., Newbolt, C. H., Ditchkoff, S. S., & Steury, T. D. (2016). Microsatellites reveal plasticity in reproductive success of white-tailed deer. Journal of Mammalogy, 97(5), 1441–1450.

Porter, W. F., Underwood, H. B., & Woodard, J. L. (2004). Movement behavior, dispersal, and the potential for localized management of deer in a suburban environment. The Journal of Wildlife Management, 68(2):247–256.

Rogers, L. L., Mooty, J. J., & Dawson, D. (1981). Foods of white-tailed deer in the Upper Great Lakes Region--a review. General Technical Report NC-65. St. Paul, MN: US Dept. of Agriculture, Forest Service, North Central Forest Experiment Station, 65.

Rudolph, B. A., Riley, S. J., Hickling, G. J., Frawley, B. J., Garner, M. S., & Winterstein, S. R. (2006). Regulating Hunter Baiting for White-Tailed Deer in Michigan: Biological and Social Considerations. Wildlife Society Bulletin, 34(2), 314–321.

Scribner, K. T., Smith, M. H., & Johns, P. E. (1989). Environmental and Genetic Components of Antler Growth in White-Tailed Deer. Journal of Mammalogy, 70(2), 284–291.

Tremblay, J.-P., Huot, J., & Potvin, F. (2007). Density-related effects of deer browsing on the regeneration dynamics of boreal forests. Journal of Applied Ecology, 44(3), 552–562.

Weber, S. J., Mautz, W. W., Lanier, J. W., & Wiley, J. E. (1983). Predictive equations for deeryards in northern New Hampshire. Wildlife Society Bulletin (1973-2006), 11(4), 331-338.

Wolch, J. R., Byrne, J., & Newell, J. P. (2014). Urban green space, public health, and environmental justice: The challenge of making cities 'just green enough.' Landscape and Urban Planning, 125, 234–244.