THE GREAT GARDEN ESCAPE: COMPARING DISPERSAL STRATEGIES OF
TWO INVASIVE VIBURNUM SPECIES

by

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Written under the direction of Dr. Claus Holzapfel and
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ABSTRACT OF THE DISSERTATION

The Great Garden Escape: Comparing Dispersal Strategies of Two Invasive Viburnum Species

By ANTHONY C CULLEN

Dissertation Director:

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Invasive species have serious ecological and economic impacts worldwide. They are known to decrease biodiversity, disrupt ecosystem functioning, and displace native species. Much of the research focused on invasive species has tried to characterizing traits of invasive species, to characterizing the communities they invade, as well as competition for resources, and lack of natural enemies. Many times, these studies occur after a species is fully entrenched and therefore hard to eradicate or control. When possible, it is critical to identify and study the next potential invasive species before they become a significant invader. My dissertation research investigates dispersal strategies of two newly invasive viburnum plant species. The focus is to understand the primary mechanisms for seed dispersal, explore spatial distribution patterns of the invasion, and to determine the genetic diversity of naturalized populations through a landscape genetics approach. By investigating \textit{V. dilatatum} and \textit{V. sieboldii} during the early stages of their invasion, my dissertation will have broad applications for land managers who may find these species in their parks by providing them with the best practices for controlling their spread. My two motivating questions are as follows: are the dispersal “strategies”
employed by closely related species comparable and what “strategy” leads to greater success at invading communities?

Chapter one investigated how fruit nutritional content influenced dispersal. We found that *V. sieboldii* fruit was dispersed during fall migration, primarily by Gray Catbirds (*Dumetella carolinensis*) and that *V. dilatatum* fruit was dispersed in the winter, mainly by American Robins (*Turdus migratorius*). The implications for dispersal were that *V. sieboldii* has a higher probability for long-distance seed dispersal than *V. dilatatum* which may explain why *V. sieboldii* is more widely distributed than *V. dilatatum*. In chapter two, we used point pattern analysis to detect local patterns of species distribution. The data suggests that *V. sieboldii* most likely relies on clonal spread at distances under three meters but is likely dispersed by birds at distances from eight to twelve meters. While *V. dilatatum* likely spreads by fruit both at distances under four meters by seedfall and by bird-enhanced seed dispersal at distances between six and thirteen meters. Chapter three explored how dispersal influences gene flow between locations. We found that all *V. sieboldii* populations freely experience gene flow and *V. dilatatum* is structured into two populations likely due to the differences in bird-enhanced dispersal. This suggest that while migratory birds are likely responsible for seed dispersal between populations of *V. sieboldii*, resident birds may have large enough ranges to disperse seeds between populations of *V. dilatatum*. This dissertation illustrates the need for a trait-based approach in context of a species natural history for assessing newly invasive species. Studies such as mine provide supporting evidence for this and also provide a baseline to ask more interesting questions about the system.
Dedication

This is dedicated to three generations of family who have fostered, supplied, and strengthened my love of nature and the environment. First to my grandfather Tony Cotroneo who fostered my love of nature by always taking me for hikes exploring the world around us. To my parents Jim and Joann Cullen who chose to live in a place with nature all around us, the Great Swamp National Wildlife Refuge. A great place to explore, make mistakes, and find my place in the larger world. Finally, to my wonderful children Evelyn and Wesley Cullen who have strengthened my connection to nature by allowing me to share and pass down the gift that was passed on to me.

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Introduction

Key areas of invasion ecology research have ranged from characterizing traits of invasive species, to characterizing the communities they invade, as well as competition for resources, and lack of natural enemies (Shea and Chesson 2002). However, since many plant invasions are context dependent (i.e. the habitat, the land use history, and the plant community composition) these approaches have yielded few generalizations to help predict invasiveness or provide solutions to land managers for control and restoration efforts (Rejmánek and Richardson 1996, Goodwin et al. 1999, Alpert et al. 2000, Daehler 2003, Moles et al. 2008). Even though, invasion biologists have difficulty finding general trends to species invasion, studies that focus on specific invaders in a defined region seem to have better success at determining invasion success (Davis et al. 2005, Stohlgren and Schnase 2006).

In many forests in the northeastern United States invasive shrubs have moved into the understory and are displacing native trees and shrubs (Silander and Klepeis 1999, Gorchov and Trisel 2003, Fagan and Peart 2004, Martin et al. 2009, Matzek 2011, Richardson and Rejmánek 2011). Many of these invasive species were brought over as horticultural specimens and have since escaped cultivation (Reichard and White 2001, Richardson and Rejmánek 2011). *Viburnum dilatatum* and *Viburnum sieboldii* (Fig. 1a and b) are two examples of horticultural plants that have become naturalized in northeastern forests. While both species are relative newcomers in the invasive plant world they are no strangers to the U.S. as they were brought over from East Asia (Fig. 1c) as ornamental specimens in the mid to late 1800s. They were valued because of their showy white flowers and attractive fruit as well as their adaptability to a wide range of
climatic and soil conditions (Rehder 1927, Dirr 1990).

Within the past thirty years both species have been expanding their range in the Northeast and some Midwest States (Fig. 2) (Early Detection and Distribution Mapping
System 2017b, a). Currently, *V. dilatatum* (Early Detection and Distribution Mapping System 2017a) is found in seven states and *V. sieboldii* (Early Detection and Distribution Mapping System 2017b) is found in twelve states according to the national map from EDDMapS. While they are not yet recognized as significant invaders, they have been noted as a local concern for invasiveness in New Jersey, New York, Pennsylvania, and Maryland (Glenn and Moore 2010, Martin and Burgiel 2012, Pennsylvania Department of Conservation and Natural Resources 2012a, b, New Jersey Invasive Species Strike Team 2017) because they displace native species.

![Figure 2: Current distribution of both species. a) Viburnum dilatatum is found in Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland and Virginia. b) Viburnum sieboldii is found in Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland, Virginia, West Virginia, Ohio, Kentucky, and Illinois.](image)

There is a huge disparity of one hundred and twenty years in the time that both species have been in cultivation until the time they started to colonize forest understories. Indeed, other successful invasive species have been shown to exhibit a similar trend where they are slow to establish until populations expand exponentially for a time. This type of invasion, referred to as lag-log because of the subsequent time between
introduction and widespread naturalization (Fig. 3) (Cousens and Mortimer 1995, Richardson et al. 2000, Crooks 2005).

Figure 3: Lag-log invasion. Certain invasive species have been theorized to show this trend when introduced into a new landscape. Adapted from “Dynamics of Weed Populations”

These two viburnum species are at a critical phase where they are just starting to colonization and spread, but are not yet widely distributed. Therefore, it is important to assess their dispersal capabilities to determine if they have the potential to become a significant invasive species. When possible, it is critical to identify and study the next potential invasive species before they become a significant invader (Regan et al. 2006, Mehta et al. 2007, Simberloff et al. 2013) for purposes of eradication. My research aims to understand these underlying mechanisms of what could potentially be the next
dominant invasive species.

My two motivating questions are as follows: are the dispersal “strategies” employed by closely related species comparable and what “strategy” leads to greater success at invading communities? The focus of this dissertation is to understand dispersal through three different lenses. Chapter one addresses dispersal of seeds by frugivorous birds by exploring fruit nutrition and the phenology of bird feeding and the potential outcomes of dispersal. The second chapter explores the distribution patterns of dispersal for both species on a local and statewide scale. The third chapter focuses on the genetic diversity of populations to explore the way in which these viburnums escaped cultivation.

By investigating *V. dilatatum* and *V. sieboldii* during the early stages of its invasion, my dissertation will have broad applications for land managers who may find these species in their parks by providing them with the best practices for controlling their spread. Also, *V. dilatatum* and *V. sieboldii* offer a rare opportunity to explore and compare the mechanisms of dispersal for non-native plants for several reasons. As congener species they are likely more ecologically similar and therefore should be more likely to have similar traits that influence dispersal strategies than those of distantly related species (Wiens et al. 2010, Burns and Strauss 2011). They share similar timelines for introduction and invasion so it is ideal to compare their rates of colonization and spread. Plus, neither species is widely distributed yet which make it feasible to utilize geospatial and molecular techniques along with empirical and experimental field research to adequately address which dispersal strategy is a bigger threat for invasibility. Therefore, they are model organisms for my dissertation project studying dispersal, distribution, and phylogenetics of invasive species.
Reference


Chapter 1: All in the timing: an investigation of the pathways to seed dispersal by birds for two invasive ornamental viburnums

Abstract
In many northeastern United States forests, invasive shrubs have moved into the understory displacing native trees and shrubs. The majority of these invasive shrub species were originally brought over as horticultural specimens and have since escaped cultivation. *Viburnum dilatatum* and *Viburnum sieboldii* are two examples of horticultural plants that have become naturalized in northeastern forests. While not yet recognized as significant invaders, in northern New Jersey they have been noted as a local concern for invasiveness. Their fruit is generally considered an attractive and reliable food source for birds and the potential exists for these species to further expand their range through bird-enhanced long-distance seed dispersal. However, despite similar fruit ripening phenology, we observed *V. sieboldii* fruit is consumed in the fall while *V. dilatatum* fruit persists into winter. We hypothesize that the difference in the timing of fruit consumption is largely driven by nutritional content of fruit, with higher nutrient fruit being eaten first. The implications for dispersal are that *V. sieboldii* may have a higher probability for long-distance seed dispersal by fall migratory birds whereas *V. dilatatum* is more likely to be dispersed locally by resident birds in the winter. To test this hypothesis, we conducted research at Lewis Morris County Park and Fosterfields Living Historical Farm in Morristown, NJ as both viburnum species are present. Avian point count surveys were conducted for each viburnum species and game cameras were set up around both viburnum communities from August-February to determine if migratory bird species (long-distance dispersers) or residents (local dispersers) were consuming fruit.
Nutritional content (energy density, percent of crude fat, and antioxidant capacity) was quantified for each viburnum fruit to explain observed differences in bird fruit preference and consumption time. We found that *V. sieboldii* fruit is mainly dispersed by Gray Catbirds (*Dumetella carolinensis*) during the fall migration and *V. dilatatum* fruit is mainly dispersed by American Robins (*Turdus migratorius*) in the winter. Therefore, we conclude that *V. sieboldii* has a higher probability for long-distance seed dispersal because it is being dispersed by migratory species in the fall whereas *V. dilatatum* is being dispersed by resident birds in the winter and therefore is only spread locally. We also found that there is a difference in the average nutritional content between the two shrubs. *V. sieboldii* fruit has higher energy density and over four times as much crude fat compared to that of *V. dilatatum* fruit. However, *V. dilatatum* fruit has over nine and a half times more antioxidant capacity than *V. sieboldii* fruit. This suggests that the timing of fruit consumption is largely driven by nutritional content of fruit, with birds selecting fruit with higher energy and fat content over fruit with relatively low energy and fat content despite high antioxidant capacity.

**Introduction**

The mutualistic relationship that exists between frugivorous birds and fruiting plants has been well-documented (Snow 1971, Wheelwright and Orians 1982, Herrera 1985, Jordano 1987); birds consume fruit for energy needed to migrate to wintering grounds or for local species to survive overwintering and plants benefit because they are able to spread their seed (Snow 1971, Thompson and Willson 1979, Baird 1980, Stiles 1980). Often seeds that are bird-dispersed have the advantage of spreading locally to potentially suitable habitats away from the parent plant or in rare cases are dispersed over
long distances (McAtee 1947, Howe and Smallwood 1982, Nathan et al. 2008). The implications of spread and colonization of new habitats can lead to a change in community composition over time, which in turn leads to changes in species and genetic diversity (Howe and Smallwood 1982, Sork and Smouse 2006, Karubian et al. 2012).

This process is largely considered beneficial to plant communities as it allows species to move and spread beyond their current habitat, increasing species richness and community resiliency (Howe and Smallwood 1982, Peterson et al. 1998, Sekercioglu 2006); however, these same processes also benefit invasive plants (Panetta and McKee 1997, Richardson et al. 2000, Gosper et al. 2005, Bartuszevige and Gorchov 2006, Buckley et al. 2006). This is problematic because, unlike natives, invasive plants have been shown to decrease biodiversity and change ecosystem functioning (Chapin III et al. 2000, Pimentel et al. 2005, Ehrenfeld 2010). Successful colonization is largely context dependent and relies on surrounding community composition, habitat connectivity, and timing of dispersal events (Robinson and Handel 1993, Nathan and Muller-Landau 2000, Nathan and Casagrandi 2004, Levey et al. 2005, Sork and Smouse 2006, Myers and Harms 2009, 2011).

Here, we will explore how the timing of dispersal events is driven by fruit nutrition and what the potential implications are for the dispersal of non-native plants. It has been shown that birds select fruit based on nutritional traits like lipid content, energy density, and antioxidant capacity. In turn, these nutritional traits can affect the timing of consumption and can impact plant dispersal (Buckley et al. 2006). For example, fruit with high lipid and high energy content (Smith et al. 2007, Cazetta et al. 2008, Smith et al. 2013) was observed to be selected first by birds during fall migration, whereas fruit that
was high in sugar and antioxidant content survived until winter when it was ultimately consumed (Cazetta et al. 2008, Greenberg and Walter 2010).

Much of the literature tries to draw distinctions between fruit nutrition and selection of fruit from native versus non-native plants but the research is mixed and has shown no general trends. Some studies have shown birds prefer non-native fruit over native fruit (Vilà and D’Antonio 1998, Lafleur et al. 2007), while another study showed a preference for native species fruit (Smith et al. 2013) and one study found no preference at all between non-natives and native species fruit (Drummond 2005). However, almost all of these studies linked fruit preference to energy and lipid content, which was always seasonally linked to either fall migration or overwintering of bird species. When examining these papers closely the common factor in selecting desirable fruit comes down to the nutritional traits associated with the species regardless of whether it is native or invasive.

So since nutritional quality of fruit can be indicative of what species’ fruit is consumed first (Johnson et al. 1985, Vilà and D’Antonio 1998, Smith et al. 2007, Greenberg and Walter 2010, Smith et al. 2013, Smith et al. 2015) and fruit phenology dictates what birds species are around when the fruit are ripe (Thompson and Willson 1979). It should then follow that fruit functional traits (nutrition and phenology) influence what bird species disperse seed and the timing of when those seeds are dispersed. Ultimately, this informs how far those seeds are likely to be dispersed. Therefore, investment in fruit nutrition becomes a strategy of life history traits that influence whether there is the opportunity for long-distance seed dispersal versus local dispersal. So, do non-native species that rely on bird dispersal exhibit different dispersal strategies
when colonizing or is there only one pathway to successful invasion?

To answer this question, we have selected two congener invasive species, *Viburnum dilatatum* and *Viburnum sieboldii*. Both species are of local concern for invasiveness in northern New Jersey however, not much is known about the mechanisms of their dispersal. This study explores how fruit nutritional traits influence the timing of fruit consumption by birds, and subsequent seed dispersal of these two viburnums. Despite being congenerers, the timing of fruit consumption by birds appear to be different {personal observation}: *V. dilatatum* fruit is consumed during the winter while *V. sieboldii* fruit is consumed during the fall even though both species’ fruits ripen around the same time. The goal is to determine if the dispersal strategies of these two species differ by examining fruit phenology and nutritional content to understand how these traits influence what bird species consume the fruit and when. This will be accomplished by: quantifying energy density, fat content and antioxidant capacity; conducting a frugivore exclusion experiment to understand fruit phenology; and determining which bird species are feeding and when by conducting avian point counts and utilizing game cameras. The overarching question is: are the dispersal strategies employed by closely related viburnum species different and what are the potential implications for invasiveness? By exploring this question, we can understand what strategy leads to greater success invading a community. Although both species fruit ripen in fall, we hypothesize that *V. sieboldii* fruit is consumed in the fall by migratory birds because it is higher in energy and fat content than *V. dilatatum* and therefore likely to be dispersed further.

**Methods**

*Study sites*
Lewis Morris County Park (park main entrance-UTM Zone 18T, 539460.76 m E, 4515753.78 m N) and Fosterfields Living Historical Farm (park entrance-UTM Zone 18T, 541795.27 m E, 4517022.97 m N) located in Morristown, in Morris County, NJ (Fig. 1) were selected for this study because both viburnum species have naturalized populations in these parks. These parks are also ideal because they are representative of the heavily invaded northeastern U.S. forest understories common in northern New Jersey.

Figure 1. Map of Lewis Morris County Park and Fosterfields Living Historical Farm.

Fruit nutrition

*Fruit collection, storage, dissection, and tissue disruption*

*Viburnum dilatatum* fruit was collected at Lewis Morris County Park during winter 2015 and again in fall 2016. *Viburnum sieboldii* fruit was collected at Lewis Morris County Park in fall 2016. Fruit for *V. dilatatum* was collected in a subpopulation near Doe Meadows (539089.20 m E, 4514898.76 m N) and fruit for *V. sieboldii* was collected in a subpopulation near Mendham Overlook (538894.35 m E, 4514572.90 m N).
Fruit was sampled randomly from shrubs within these subpopulations. Per shrub, seven to ten fruits were harvested per cyme from multiple cymes on the shrub. In order to quantify energy density, percent crude fat, and antioxidant capacity, approximately 454 g of fruit was collected per species. Fruit was placed in a resealable freezer bag, labeled with species, date, and location before being transported back to the lab. In the lab, fruit were washed and any stems or twigs were removed. Processed fruit was placed in a -20°C chest freezer (Coldspot, Sears Roebuck and Company, Hoffman Estates, Illinois, USA) for storage. Fruits were thawed and dissected to remove the seed from the pulp. Fruit was dissected in an aluminum weigh pan to keep pulp and skin contained when removing the seed. Fruit in the pans were weighed on AB104-S balance (Mettler-Toledo, Columbus, Ohio, USA). After dissection the pan number, species, collection date, and sites were recorded on a datasheet. Samples were then placed in a 650G Isotemp drying oven (Fisher Scientific, Pittsburgh, Pennsylvania, USA) at 50°C and dried for about three days. After three days, samples were reweighed until mass from water loss had stabilized. Fruit that were fully dried were scraped from the aluminum pan and were then placed into a mortar and ground down into a powder. Liquid nitrogen was added to help grind the fruit sample. The powdered (homogenized) fruit sample was placed in a scintillation vial and sent off for fruit nutritional analysis. Fruit energy density (kJ/g dry mass), percent crude fat (dry mass), and antioxidant capacity from Folin (mg phenol GAE/g dry mass) and Trolox equivalent antioxidant capacity (µg/mg dry mass) was quantified by Susan Smith-Pagano at Rochester Institute of Technology in Rochester, NY with three trials per attribute. These fruit nutrition attributes have shown to be important in the timing and

**Energy density**

Energy density was quantified from approximately 1 g of dried and homogenized fruit from each sample. The sample was pressed into a pellet and then analyzed in a Parr 1341 bomb calorimeter for *Viburnum dilatatum*, winter 2015 and *Viburnum sieboldii*, fall 2016 and a Parr 6100 bomb calorimeter for *Viburnum dilatatum*, fall 2016, using a Parr 1108 oxygen bomb vessel (Parr Instrument Company, Moline, Illinois, USA). Samples were ignited with either 10 cm of ignition wire (Parr 1341 system) or cotton thread (Parr 6100 system) and temperature change after ignition in the vessel was measured with 2000 g of water in the water jacket. Energy density in kilojoules per gram was then calculated using a 1.0 g benzoic acid standard pellet. Both bomb calorimeters are comparable to one another because they both use the same bomb vessels and are calibrated with the benzoic acid standard before each run. The difference between the two bomb calorimeters are that the Parr 6100 has more automated features than the Parr 1341.

**Percent crude fat**

Percent dry mass of fat was measured in dried, homogenized pulp plus skin of the viburnum fruit. Approximately 1 g of sample was weighed into an Ankom XT4 sample bag (Ankom Technology, Macedon, New York, USA) and then heat sealed. Bags were pre-dried overnight at 102°C and then extracted in an Ankom XT-10 fat extractor for 60 min at 90°C in petroleum ether. Percent dry mass fat was calculated as mass lost from the bag after extraction and subsequent drying at 102°C for 1 hour.

**Antioxidant capacity: Folin and Trolox equivalent antioxidant capacity**
Folin and Trolox equivalent antioxidant capacity (TEAC) are measures of the fruit total antioxidant capacity. Folin and TEAC were determined via ABTS analysis adapted from Magalhães 2010 (Magalhães et al. 2010). To extract antioxidants from the fruit approximately 30 mg of freeze-dried viburnum drupes of each sample were placed in 1.5 mL centrifuge tubes. Next, 1.5 mL of 80% v/v methanol with 0.5% acetic acid was added to each tube. Tubes were sonified for 30 minutes, centrifuged for 10 minutes and the supernatant was then filtered through a 0.22 µm syringe filter (nylon membrane). Extracts were stored at -20°C until analysis.

To quantify the amount of folin per sample, a stock solution of gallic acid (Sigma-Aldrich, St. Louis, Missouri, USA) was prepared to 1000 ppm in Nanopure water. From the stock, working standards of 40, 30, 20, 10, 6, 3 ppm were prepared. A 0.35 M sodium hydroxide solution was prepared in water. A 1:5 v/v dilution of the Folin-Ciocalteu reagent was also prepared with water. For the microplate analysis, water and the extraction solvent were used as blanks.

Fifty µL of sample, standard, and blank were added to the microplate. To each well, 50 µL of the 1:5 v/v folin reagent (Sigma-Aldrich, St. Louis, Missouri, USA) was added and allowed to oxidize for at least 1 min. Finally, 100 µL of 0.35 M sodium hydroxide was added to each well and the plate was read at 760 nm within 3 mins of base addition.

To quantify the amount of TEAC per sample an ABTS solution was prepared to be 7.0 mM and a potassium persulfate solution was prepared to be 2.45 mM (both in Nanopure); equivalent volumes of the ABTS solution and pot. Persulfate (Sigma-Aldrich, St. Louis, Missouri, USA) were added to a new 50 mL volumetric flask and this “ABTS
radical” solution was allowed to react, in the dark, for 15 hours. The following day, a 1.0 mM Trolox stock solution was prepared in 50% ethanol (Sigma-Aldrich absolute EtOH). From the stock, working standard were prepared in 50% EtOH to concentrations of 50, 30, 20, 10, 5, 2.5 µM.

Prior to testing, the ABTS radical solution was diluted with water to give an absorbance of 0.800 ± 0.050 at 734 nm under experimental conditions (e.g. 150 µL ABTS radical + 150 µL of water). A dilution of 1:17 gave such an absorbance. To each well, 150 µL of sample, standard, and blank were placed in the microplate. Next, 150 µL of the 1:17 dilution of ABTS was added to each well and the plate was immediately inserted into the reader. Temperature within the reader was held constant at 25ºC. Readings at 734 nm occurred every minute for 15 minutes. The ABTS results used the 15 minute kinetic end-point.

**Frugivore exclusion experiment**

To understand the timing of fruit loss and to quantify whether that loss was due to consumption versus abscission, infructescences on shrubs were experimentally manipulated with two treatment types: enclosed or unenclosed. Fifteen shrubs per species were selected and two infructescences on each shrub with similar numbers of fruit per cyme were chosen. Both cymes were marked with different colored chenille stems to indicate treatment; the unenclosed treatment remained unaltered (Fig. 2a) while the enclosed treatment was covered by a 15.24x22.86 cm white Organza bag (Fig. 2b) to prevent feeding. This experiment was conducted from August 5, 2016 to February 27, 2017. Number of fruit per cyme for each treatment, for all shrubs, were counted weekly during this time to account for changes in number of fruit per cyme.
To determine weekly fruit loss per treatment, the total number of fruit was summed across all shrubs (n=15) for every week of the frugivore exclusion experiment. However, because the total number of fruit was not equal among treatments and across species the fruit loss need to be converted into a weekly relative percent. The total number of fruit remaining from the current week was divided by the total number of fruit from the previous week, then that percent was subtracted from one to get the relative percent difference of fruit loss per treatment per week. To further explore the relationships between consumption versus abscission, the difference of the weekly relative percent of fruit loss from the enclosed treatments (n=15), (presumably from abscission) by weekly relative percent of fruit lost from the unenclosed treatment (n=15) (combination of abscission and consumption) calculated the estimated weekly relative percent consumption. The following formulas display weekly relative percent of the fruit loss per treatment and the estimated weekly relative percent of consumption.

\[ 1 - \frac{U_t}{U_{t-1}} = Un_{wrpl} \]  

\[ 1 - \frac{E_t}{E_{t-1}} = En_{wrpl} \]  

\[ CONSUMP = Un_{wrpl} - En_{wrpl} \]

where \( U \) represents the unenclosed treatment, \( E \) represents the enclosed treatment, \( t-1 \) is the total fruit from the previous week, \( t \) is the total fruit from the current week, \( Un_{wrpl} \) is the unenclosed weekly relative percent fruit loss and \( En_{wrpl} \) is the enclosed weekly relative percent fruit loss, and \( CONSUMP \) represents the estimated weekly relative percent of consumption. If \( CONSUMP \) is a negative value there was more fruit loss to abscission, whereas if \( CONSUMP \) is a positive value more fruit was loss to
consumption than abscission. These methods are adapted and modified from Smith et al. papers (Smith et al. 2007, Smith et al. 2013).

![Treatment types for frugivore exclusion experiment](image)

Figure 2. Treatment types for frugivore exclusion experiment a) an unenclosed treatment marked by a red chenille stem and b) an enclosed treatment Organza bag marked by an orange chenille stem.

**Avian point counts**

Avian point counts were employed for both viburnum species to survey the birds that fed on viburnum fruit. Multiple seasons of avian point counts were conducted with ornithologist Kathleen Farley at fixed locations from summer 2015 to winter 2017. Monitoring started when fruits first ripened until fruits were almost totally absent from the shrubs, which fluctuated from year to year. Point counts were conducted at sunrise during the fall and delayed until two hours after sunrise in the winter due to freezing temperatures. Point counts ran for ten minutes per location, during which bird species actively visiting and feeding on viburnums were recorded. Locations were selected based on where populations of viburnum species were found but the number of shrubs per location varied as it was difficult to standardize number of shrubs per location.
For *Viburnum dilatatum*, the first year there were a total of six avian point count surveys at four locations in Lewis Morris County Park. Four point counts were conducted weekly in the fall from October 16, 2015 to November 6, 2015 and then two more were done in the winter on December 21, 2015 and February 17, 2016. Point counts were switched over from weekly surveys to monthly during the first year because of the slow rate of removal of fruit (monthly surveys were maintained for the second year). The second year there were a total of six avian point counts at six locations in Lewis Morris County Park (Fig. 3) conducted monthly from September 15, 2016 to February 20, 2017. Two more points were added in the second season to ensure thorough sampling effort throughout the park.

Figure 3. The six point count locations for *Viburnum dilatatum*. Points 1-4 (All- UTM Zone 18T; 1-538915.11 m E, 4514617.52 m N; 2- 538878.46 m E, 4514895.64 m N; 3- 539134.30 m E, 4515091.13 m N; 4- UTM 539262.89 m E, 4515467.64 m N) were conducted the first season and points 5 and 6 (both- UTM Zone 18T; 5- 539064.56 m E, 4514870.18 m N; 6- 539135.15 m E, 4515015.55 m N) were added for the second season.
For *Viburnum sieboldii*, the first year there were a total of three avian point count surveys at four locations at Lewis Morris County Park and Fosterfields Living Historical Farm conducted weekly from August 27, 2015 to September 18, 2015. For the second year there were a total of five avian point counts at six locations in Lewis Morris County Park and Fosterfields Living Historical Farm (Fig. 4) conducted weekly from August 5, 2016 to August 30, 2016. Two more points were added in the second season to ensure thorough sampling effort throughout the park.

![Figure 4. The six point count locations for *Viburnum sieboldii*. Points 1-4 (All- UTM Zone 18T; 1-538941.15 m E, 4514569.45 m N; 2- 539228.23 m E, 4515388.93 m N; 3- 541852.17 m E, 4-541461.20 m E, 4516867.41 m N) were conducted the first season and points 5 and 6 (both- UTM Zone 18T; 5- 538930.30 m E, 4514497.11 m N; 6- 539086.28 m E, 4514322.09 m N) were added for the second season.](image)

**Game cameras**

Game cameras were installed throughout Lewis Morris County Park near fruiting populations of each viburnum species to capture animals feeding on viburnum fruit to
help supplement point count data. Game cameras locations were selected based on an abundance of fruiting cymes and the locations of the cameras frequently changed once all fruit was removed or abscised. Monitoring started when fruits first ripened until when fruits were almost totally absent from all shrubs, which fluctuated from year to year. Data from game cameras was collected weekly and analyzed for presences of animals visiting or feeding on viburnum fruit.

For *Viburnum dilatatum*, the first year there was one SPYPOINT HD-7 trail camera (Swanton, Vermont, USA) installed at Lewis Morris County Park from October 16, 2015 to February 17, 2016. The second year there were a total of three SPYPOINT Force-10 trail cameras installed at Lewis Morris County Park from August 2, 2016 to February 27, 2017.

There were no game cameras installed the first year for *Viburnum sieboldii*. However, for the second year there were a total of four SPYPOINT trail cameras installed at Lewis Morris County Park. Three HD-7 trail cameras were installed from August 2, 2016 to September 29, 2016. Another camera, SPYPOINT Force-10 trail camera (Swanton, Vermont, USA), was added from August 27, 2016 through September 15, 2016 to ensure thorough sampling effort throughout the park during peak feeding time for *Viburnum sieboldii*.

**Data analysis**

Fruit nutritional analysis was run for three trials for each fruit attribute: fruit energy density (kJ/g dry mass), percent crude fat (dry mass), Folin (mg phenol GAE/g dry mass), and TEAC (µg/mg dry mass). These trials were averaged and standard deviations were calculated and presented in tabular form. All raw data for the frugivore
exclusion experiment, avian point count, and game cameras was analyzed in SigmaPlot (SigmaPlot version 11.0, Systat Software, Inc., San Jose, California USA, www.sigmaplot.com). Survival analyses for frugivore exclusion experiment was conducted in RStudio (RStudio Team 2016) using the packages survival (Therneau 2018b) to determine the probability of weekly fruit survival per species per treatment with a Kaplan-Meier survival curve and coxme (Therneau 2018a) to determine the relative risk of fruit loss over time with species and treatment as fixed effects and the infructescence cymes nested with the shrub as a random effect with Cox mixed effects model. Survival analyses accounted for the effects of time in weeks (n=12 for Viburnum sieboldii and n=24 for Viburnum dilatatum), treatments (n=2), and shrubs (n=15) per species. Avian point count and game camera data were compiled for both species in SigmaPlot by the total number of visits and feeds. All maps were created in QGIS (Quantum GIS Development Team 2017, Quantum GIS Geographic Information System, Open Source Geospatial Foundation Project, http://qgis.osgeo.org) and edited or modified in Inkscape (Harrington, B. et al 2004-2005, Inkscape, https://inkscape.org/en/). Base layer for the state of New Jersey and its counties was provided by The State of New Jersey, Department of Environmental Protection, Bureau of GIS and boundary maps provided by Janice Karmon, GIS Coordinator, Park Planning & Development for the Morris County Park Commission.

Results

Fruit nutrition

Viburnum sieboldii fruit from fall 2016 had slightly more energy density and more than four times the amount of percent crude fat than fall 2016 Viburnum dilatatum
fruit (Table 1). However, fall *V. dilatatum* fruit had over five and a half times more folin and nine and a half times the amount of TEAC. *V. dilatatum* fruit collected winter 2015 had slightly less energy density and percent crude fat than *V. dilatatum* fruit collected the next year, fall 2016. Interestingly, *V. dilatatum* fruit from winter 2015 also had two and a half times less folin and three and a half times less TEAC than *V. dilatatum* fruit from fall 2016.

Table 1. Average and standard deviation* for the three trials of the homogenized fruit samples for the nutrition analysis for energy density (kJ/g dry mass), percent crude fat (dry mass), folin (mg phenol GAE/g dry mass), and TEAC (µg/mg dry mass) for *Viburnum dilatatum* winter 2015, fall 2016 and *Viburnum sieboldii* fall 2016. *Standard deviation accounts for variability in trials not biological variability in fruit.

<table>
<thead>
<tr>
<th>Species/Season</th>
<th>Energy Density (kJ/g dry mass)</th>
<th>% crude fat (dry mass)</th>
<th>Folin (mg phenol GAE/g dry mass)</th>
<th>TEAC (µg/mg dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Viburnum dilatatum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter 2015</td>
<td>15.91±0.18</td>
<td>1.98±0.13</td>
<td>18.42±2.81</td>
<td>18.86±2.74</td>
</tr>
<tr>
<td>Fall 2016</td>
<td>18.06±0.05</td>
<td>3.07±0.21</td>
<td>46.40±3.51</td>
<td>65.39±12.62</td>
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<tr>
<td><em>Viburnum sieboldii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall 2016</td>
<td>21.43±0.31</td>
<td>13.41±0.21</td>
<td>8.13±0.58</td>
<td>6.87±0.67</td>
</tr>
</tbody>
</table>

*Frugivore exclusion experiment*

*Viburnum dilatatum* started off the frugivore exclusion experiment with a total of 1,039 fruit for the unenclosed treatment and 1,093 fruit for the enclosed treatment for all 15 shrubs. By the end of the experiment, the unenclosed treatment had a total of 68 fruit and the enclosed treatment had a total of 413 fruit (Fig. 5-red lines). The average fruit per shrub plus the standard deviation, regardless of treatment, was 71.07±27.89. *Viburnum sieboldii* started off the frugivore exclusion experiment with a total of 318 fruit for the unenclosed treatment and 337 for the enclosed treatment for all 15 shrubs (Fig. 5-purple lines). By the end of the experiment both treatments had lost all of their fruit. The average fruit per shrub plus the standard deviation regardless of treatment was 21.83±5.05.

The Kaplan-Meier survival analysis for *V. dilatatum* determined that the median
survival time of the unenclosed treatment was seven weeks compared to twenty weeks for enclosed treatment (Fig 6). For *V. sieboldii* fruit the median survival time of the unenclosed treatment was two weeks compared to seven weeks for the enclosed treatment (Fig 6). There was a significant difference between fruit survival between unenclosed and enclosed treatments for both species with a log-rank test of \( p < 0.001 \). The Cox mixed effects model determined that *V. sieboldii* had a seven and a half times higher risk than *V. dilatatum* of fruit loss and the unenclosed treatment, regardless of species, has approximately nine and a half times higher risk of fruit loss than enclosed treatment, for both fixed effects, the difference between species, and treatments were significantly different, \( p < 0.001 \). When considering the random effects of shrub on the risk for fruit loss, it was five and a half times more than the norm and for cyme nested in shrub, the risk of fruit loss was nearly one and a half times more than the norm.
Figure 5. These figures represent total weekly fruit per treatment (n=2) for all shrubs (n=15) for *Viburnum dilatatum* (red lines) and *Viburnum sieboldii* (purple lines). *V. dilatatum* had more fruit per cyme than *V. sieboldii*. However, *V. sieboldii* fruit was consumed faster and more completely than its counterpart *V. dilatatum*, which persisted on the shrub until the end of February.
Figure 6. Probability of weekly fruit survival per treatment for *Viburnum dilatatum* (red lines) and *Viburnum sieboldii* (purple lines). *V. dilatatum* unenclosed had a median survival of 7 weeks where the enclosed had a median survival of 20 weeks. *V. sieboldii* unenclosed had a median survival of 2 weeks where the enclosed had a median survival of 7 weeks.

To determine fruit loss to consumption the weekly relative percent was calculated for both species. *Viburnum dilatatum* was consumed early in the season from August to October although the largest spike of consumption occurred in February (Fig 7a). Otherwise from November until January the relative percent difference fluctuated between little consumption to negative consumption (fruit loss to abscission). However, *Viburnum sieboldii* was largely consumed in August and September with a smaller spike in October before all fruit was lost (Fig 7b).
Figure 7. The estimated weekly percent consumption a) *Viburnum dilatatum* and b) *Viburnum sieboldii*. *V. dilatatum* fruit was consumed early in the fall and then again in mid-February. *V. sieboldii* fruit was consumed mainly in August and September with a brief peak in early October.

**Avian point counts and game cameras**

The combination of avian point count surveys and game cameras showed that *Viburnum dilatatum* fruit’s primary avian consumer were American Robins (*Turdus migratorius*) (Table 2a and Fig. 8a) in February of 2016 and 2017 (Fig 9a). White-throated Sparrows (*Zonotrichia albicollis*) were the second greatest avian consumer of *V. dilatatum* fruit but only were the third most frequent visitor (Table 2b and Fig. 8b) in December of 2016 (Fig 9a). All observed avian feeding took place in the winter however, six other bird species were observed visiting the shrub fall of 2015 and 2016, none were seen feeding. These species included Tufted Titmouse (*Baeolophus bicolor*), Black-capped Chickadee (*Poecile atricapillus*), Eastern Towhee (*Pipilo erythrophthalmus*), Gray Catbird (*Dumetella carolinensis*), Hermit Thrush (*Catharus guttatus*), and Northern Parula (*Setophaga americana*) with two visits per species except for Tufted Titmice, which accounted for the second most frequent visitor to *V. dilatatum* with four visits (Table 2a and Fig 8a). When consider avian feeding only, American Robins accounted for 71.43% and White-throated Sparrows accounted for 28.57% (Fig. 10a). While not
captured during avian point counts, the game cameras showed that the primary mammal and overall consumer of *V. dilatatum* fruit was the Eastern Chipmunk (*Tamias striatus*) August through October of 2016, followed by the Eastern Gray Squirrel (*Sciurus carolinensis*) in August 2016 and again in February 2017 (Table 2a and Fig. 9a).

Table 2. Taxa and species that visited and fed for a) *Viburnum dilatatum* and b) *Viburnum sieboldii*. The main avian species that consumed *V. dilatatum* fruit was the American Robin and main avian mammal species were the Eastern Chipmunk and Eastern Gray Squirrel. No mammals were observed on *V. sieboldii*, the main avian consumer was the Gray Catbird.

<table>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Total</td>
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Figure 8. Total number of avian visits and feeds for both field seasons. Data includes avian point count and game cameras. a) *Viburnum dilatatum* is primarily consumed by American Robins, followed by White-throated Sparrows and b) *Viburnum sieboldii* is primarily consumed by Gray Catbirds, as well as by Baltimore Orioles, Northern Mockingbirds, Tufted Titmice, Northern Cardinals, and a Red-bellied Woodpecker.
Figure 9. The timing of the total number of feeds for all taxa across field seasons. Data includes avian point count and game cameras. a) *Viburnum dilatatum* is primarily consumed in the fall by the Eastern Chipmunk and the Eastern Gray Squirrel while American Robins and White-throated Sparrows along with the Eastern Gray Squirrel consume fruit in winter. b) *Viburnum sieboldii* is primarily consumed by Gray Catbirds in August and September. Baltimore Orioles, Northern Mockingbirds, Tufted Titmice, Northern Cardinals, and a Red-bellied Woodpecker were observed to consume fruit in August 2016.

Figure 10. Percent of bird feedings per shrub for both field seasons. Data includes avian point count and game cameras a) American Robins make up 71.43% and White-throated Sparrows 29% of the avian diet for *Viburnum dilatatum* 2015-2017 field seasons. b) Gray Catbirds 71%, Baltimore Orioles 7%, Northern Mockingbird 6%, Tufted Titmice 6%, Northern Cardinal 7%, Red-bellied Woodpecker 3% of the avian diet for *Viburnum sieboldii* 2015-2016 field seasons.
make up the remaining 28.57% of birds that feed on *Viburnum dilatatum* fruit while b) Gray Catbirds make up 70.97%, Baltimore Orioles, Northern Mockingbirds, Tufted Titmice, and Northern Cardinals make up 6.45%, and Red-bellied Woodpeckers are 3.23% of birds that feed on *Viburnum sieboldii*. 

Based on the observations from the avian point count surveys and game cameras, *Viburnum sieboldii* fruit was only consumed by birds in August and September. The primary consumer and visitor were Gray Catbirds (*Dumetella carolinensis*) (Table 2b and Fig. 8b) both in August 2015 and August and September 2016 (Fig. 9b). Baltimore Orioles (*Icterus galbula*), Northern Mockingbirds (*Mimus polyglottos*), Tufted Titmice (*Baeolophus bicolor*), Northern Cardinals (*Cardinalis cardinalis*) all had equal number of visits and feedings in August 2016, while Red-bellied Woodpecker (*Melanerpes carolinus*) accounted for two visits and only one feeding (Table 2b and Fig. 8b) in the same month (Fig. 9b). For feeding only, Gray Catbirds accounted for 70.97%, Baltimore Orioles, Northern Mockingbirds, Tufted Titmice, Northern Cardinals each accounted for 6.45% and Red-bellied Woodpecker accounted for 3.23% (Fig. 10b). While four other species were observed visiting the shrub, none were seen feeding. These species included Cedar Waxwing (*Bombycilla cedrorum*), Common Grackle (*Quiscalus quiscula*), Ovenbird (*Seiurus aurocapilla*), and Wood Thrush (*Hylocichla mustelina*) with one visit per species (Table 2b and Fig. 8b). 

**Discussion**

**Fruit nutrition**

*Viburnum sieboldii* fruit had more energy density and more than four times the amount of percent crude fat than fall *Viburnum dilatatum* fruit. Fall *V. dilatatum* fruit had over five and a half times more folin and nine and a half times the amount of TEAC (Table 1). *V. sieboldii* fruit was consumed in August and early September during fall
migration while *V. dilatatum* fruit persisted until winter despite the fact that both species generally ripen around the same time. This suggests that energy density and especially fat content are important selection factors for migrating birds while antioxidant content may not be as integral. Smith et al. (2007) found in their study assessing fruit quality for fall migratory songbirds that fruit with high percent fat and energy density were selected first. Smith et al. also had similar findings in their 2013 and 2015 papers that fruit with high percent fat and energy density were selected first. While these latter papers also focused on comparing nutrition content for native versus invasive fruit, it still validates the idea that fruit with comparatively high fat and energy content will be selected over fruit with low fat and energy during fall migration. Smith et al. (2007 and 2013) also speculate that fruits that persist may do so because of higher content of secondary compounds that could affect digestion or gut retention. Although, Smith et al. (2015) goes on to note that secondary compounds may be beneficial for birds experiencing oxidative stress due to migration. Indeed, Jenni-Eiermann et al. (2014) showed that migratory birds do experience oxidative stress during extended flights. Consumption of fruit high in antioxidants could mediate this stress. When considering only antioxidant properties, Bolser et al. (2013) find that fruit, including *Viburnum dentatum* (arrowwood viburnum), with high anthocyanin and antioxidant capacity were consumed at faster rates than those with low content anthocyanin despite high total antioxidant capacity. Evaluating a greater suite of factors, Smith et al. (2007, 2013, and 2015) found *V. dentatum* to be selected for its high energy and fat content and not for antioxidants alone. What Bolser et al. found in their 2013 paper might be more easily explained by Schaefer et al. (2008), where birds use anthocyanin color as a foraging signal for antioxidant reward. It would also explain
why other fruit that Bolser et al. investigated that were low in total anthocyanin but high in antioxidant capacity such as oriental bittersweet (*Celastrus orbiculatus*), multiflora rose (*Rosa multiflora*), and winterberry (*Ilex verticillata*) were selected later. Alan et al. (2013) also concluded that antioxidants alone were not the complete reason *V. dentatum* fruit was selected and that fat content was a factor. Like Bolser et al. (2013), Alan et al. (2013) found the same fruits that persisted and consumed less were multiflora rose and oriental bittersweet, which Alan attributed to carotenoid content. Again, Schaefer et al. (2008) may provide insight, as their study showed that birds could not use color as a determination for carotenoid content (only anthocyanin content) of fruit and therefore could not use it as a selection factor. Schaefer et al. also found that anthocyanin content correlated with energy content, but there was no correlation with carotenoid and total energy.

These findings are applicable to the study of these two invasive viburnums. *V. sieboldii* has a dark purple fruit which implies that it could have high anthocyanin content. While that was not explicitly measured in this study it may be a factor in fruit selection. In contrast, *V. dilatatum* is a red fruit which implies it could have high carotenoid content. The fact that *V. dilatatum* had low energy and fat, was high in antioxidants and perhaps carotenoids may all be reasons why it was not selected over *V. sieboldii*.

**Frugivore exclusion experiment**

The nutritional data provides the reasoning as to why birds may select one species’ fruit over another, but the frugivore exclusion experiment illustrates the rate of consumption and survival of those fruit throughout the season differ drastically between the species. For *V. sieboldii*, the fruits were consumed faster than those of *V. dilatatum*,
which persisted until winter (Fig. 5). For *V. sieboldii* peak consumption was in August with the highest weekly relative percent of consumption taking place in the 3rd week (Fig 7b). While *V. dilatatum* showed early consumption from August through November, there was more fruit lost to abscission in December and late January with a small peak for consumption in early January and the highest weekly relative percent of consumption taking place in the 23rd week of the experiment in February (Fig 7a). The survival analysis also supports that *V. sieboldii* was consumed 7.5 times faster than *V. dilatatum* (Fig. 6).

Other studies that measured consumption rates, like Smith et al. (2007 and 2013), linked the rates of consumption to fat and energy content. Interestingly, Greenberg and Walter (2010) found that mean fruit survival time was positively correlated with total sugar and negatively correlated with fat content. While total sugar was never specifically measured in this study, sugar content may be another reason to explain differences found in consumption between the high fat content *V. sieboldii* versus the high antioxidant and potentially high sugar content of *V. dilatatum*. This would not be unheard of as the native *Viburnum opulus* L. var. americanum Aiton (American cranberrybush) and the closely related *Viburnum opulus* L. var. *opulus* (European cranberrybush) fruit shares some of the same traits as an invasive *V. dilatatum* fruit; as birds do not prefer it over other fruit, it typically isn’t consumed until the winter and potentially has high sugar content (Jones and Wheelwright 1987, Witmer and Van Soest 1998, Witmer 2001, Drummond 2005). Meanwhile, *V. sieboldii* shares some of the same traits to another native, *V. dentatum*, as it has high energy and high lipid content and is also consumed during fall migration (Smith et al. 2007, Smith et al. 2013).
Avian point count and game cameras

Our results show that the primary avian consumers of *V. dilatatum* fruit are American Robins and that the overall primary consumer are the Eastern Chipmunk and the Eastern Gray Squirrel (Table 2a). While Gray Catbirds are the primary consumers of *V. sieboldii* fruit (Table 2b and Fig. 8b). The species associated with consumption and the variation in the timing of fruit consumption is supported by point counts, game cameras, and the frugivore exclusion experiment. Regardless of species or the year the survey was conducted (2015 or 2016), *V. sieboldii* fruit was mainly consumed in August during the start of the fall migration. However, *V. dilatatum* fruit was consumed in August through October and then again during the winter from December through February.

Avian point counts in 2015 observed that Gray Catbirds were the only species visiting (n=4) and feeding (n=2) on *V. sieboldii* fruit exclusively in August. The 2016 avian point counts for *V. sieboldii* yielded more support for this trend of Gray Catbirds visiting (n=17) and feeding (n=11) in August. In addition, with 2016 data from game cameras, Gray Catbirds were seen visiting (n=19) and feeding (n=9) in August and September (Fig. 9b). This data lends overall support for Gray Catbirds being the primary dispersers of *V. sieboldii* fruit.

Other bird species observed in the 2016 avian point count for *V. sieboldii* were Baltimore Orioles, Northern Mockingbirds, and Tufted Titmice with equal amounts of visits (n=2) and feed (n=2) in August. In addition, 2016 data from game cameras captured Northern Cardinals visiting (n=2) and feeding (n=2), along with a juvenile Red-bellied Woodpecker visiting (n=2) and feeding (n=1) in August (Table 2b, Fig. 8b, and Fig 9b). While Cedar Waxwing, Common Grackle, Ovenbird, and Wood Thrush were
observed visiting the shrub (n=1 per species) they were never seen actively feeding (Table 2b and Fig 8b).

Out of the six species that were observed feeding on *V. sieboldii* fruit, Gray Catbird, Baltimore Oriole, and Northern Cardinal are migratory species while the Tufted Titmouse, Northern Mockingbird and Red-bellied Woodpecker are generally considered year-round residents in New Jersey forests (Stiles 1980, Rodewald 2017). The timing of fruit consumption and the species consuming the fruit suggest that *V. sieboldii* seed will be dispersed locally but has the probability for long-distance seed dispersal because migratory species consume the fruit in August and September. We know that the primary consumers, Gray Catbirds, have been shown to migrate to wintering grounds during August and September (Thobaden Jr. et al. 1987, Malmborg and Willson 1988, Ryder et al. 2011). Although Gray Catbirds can be year-round residents along the east coast (Smith et al. 2011) it is not unreasonable to assume that due to the timing of consumption that a proportion of Gray Catbirds consuming *V. sieboldii* fruits would be migrants.

Avian point counts in 2015 for *V. dilatatum* provided no insight as what species consumed fruit as only Tufted Titmice (n=4) and Black-capped Chickadee (n=1) were observed visiting the shrub in October. A game camera provided the only conclusive feeding data during the 2015-2016 season that captured American Robins visiting (n=2) and feeding (n=1) in February 2016 (Fig. 9a). The 2016-2017 avian point counts for *V. dilatatum* again provided no insight as what species consumed fruit as only one Gray Catbird and Eastern Towhee were observed visiting the shrub in September. However, the 2016-2017 data from game cameras captured American Robins visiting (n=12) and feeding (n=4) in January (n=3 visit) but primarily in February (n=9 visits and n=4 feeds)
(Fig. 9a). This data, solely from the game cameras lends overall support for American Robins being the primary avian dispersers of *V. dilatatum* fruit.

The only other bird species observed in the 2016-2017 game camera data that was actively feeding on *V. dilatatum* fruit were White-throated Sparrows (n=2 visits, n=2 feeds) (Table 2a and Fig. 8a). A Hermit Thrush and Northern Parula were also captured by the game cameras visiting the shrub (n=1 per species) during the 2016-2017 season, but they were never seen actively feeding. Although both American Robins and White-throated Sparrows are migratory birds (Falls and Kopachena 2010, Vanderhoff et al. 2016) the timing of feeding for both species was in winter; January and February for American Robins and December for White-throated Sparrows. This suggest that perhaps these individuals were winter residents. If so, *V. dilatatum* seed will most likely to be dispersed locally and has little chance for long-distance seed dispersal. We know that the primary avian consumers, American Robins, have been shown to migrate between October and November (Thobaden Jr. et al. 1987, Malmborg and Willson 1988, Vanderhoff et al. 2016) and return in the spring around March (Jones et al. 2012, Vanderhoff et al. 2016). American Robins feeding appeared to be very ephemeral in nature with peak consumption time in February. Anecdotal evidence from the game cameras suggest that Americans Robins peak consumption time in February for both survey years also coincided after a snowfall when potential food resources may have been limited. This may explain why avian point counts never detected their presence; only game cameras were successful at documenting feeding because American Robins seemed to feed all at once and then move on rather than slowly consuming the fruit over a longer time period.
As stated, *V. dilatatum* was not observed to be consumed in the fall by any bird species, but rather in the winter. However, the absolute number of fruit loss (Fig. 5) show that half of the fruit is lost by October and the relative percent of fruit loss (Fig. 7a) show that fruit was consumed between August and September. However, the game cameras were able to detect what our point counts could not, that Eastern Chipmunks and Eastern Squirrels were responsible for fruit consumption during this time. Eastern Chipmunks accounted for a total of sixteen visits to fruit cymes and fourteen feeds (Table 2a), all of which happened from August through October (Fig. 8a) during the 2016-2017 season when the fruit was either blushing or red. Eastern Squirrels accounted for a total of thirteen visits to fruit cymes and twelve feeds (Table 2a). Nine visits and feeds took place in August while the fruit was still green while four visits and three feeds took place in February when the fruit was red and shriveled (Fig 8a).

Unlike birds, which were observed to feed on a fruit or two per cyme before moving on to the next cyme, both rodent species were captured on the game cameras removing whole cymes at a time. This is presumably why there was a large drop in total fruit early on. Eastern Chipmunks and Eastern Squirrels are considered to be seed predators and therefore are not likely to be seed dispersers (Janzen 1971). Although both species are known to cache seeds which can be a means for seed dispersal, we have found no literature that supports the idea that either species are known to cache fleshy fruit like *Viburnum spp.* but rather rodents such as chipmunks or squirrels are known to consume fleshy fruit and only cache larger seeds and nuts like that of the acorn (Moller 1983, Vander Wall 2010). Kollmann et al. (2000) study about seed predation for fleshy fruit supports this argument as they found that in Europe, *Viburnum lantana* had one of the
highest rates of seed removal compared to other fleshy fruit, but they speculate that because of the seed remnants found near feeding sites that caching was unlikely. Kollmann et al. (2000) also found that seed predation by rodents was highest in summer, not unlike our study. Therefore while both Eastern Chipmunks and Eastern Squirrels consumed more fruit than the American Robin, it is unlikely these seeds are being dispersed.

Another explanation as to why rodents consume *V. dilatatum* fruit during fall but birds wait until winter could be antioxidant capacity. The nutrition analysis for *V. dilatatum* from winter 2015 and fall 2016 (Table 1) suggest that there is a dramatic drop in folin and antioxidant content between fall and winter, when fruit become ripe in September and when fruit start to shrivel in December. This is not conclusive evidence that antioxidant capacity definitively drops in the fruit because the fruit was harvested in different years and is therefore not comparable. It could be that there was yearly difference in fruit crop nutrition. However, it does suggest antioxidant capacity may diminish over time which could make the fruit more palatable to birds seeking out fruit in the winter. This may explain why only rodents are found consuming the fruit early on and why birds may wait until winter when antioxidant content is reduced before feeding. Both Herrera (1982a) and Cipollini et al. (1997, 2000) have stated that secondary compounds protect unripe fruit and may discourage birds from feeding until the fruit are ripe and viable for dispersal. It is not clear why chipmunks and squirrels would consume unripe *V. dilatatum* fruit, but regardless this behavior was observed on the game camera and most likely accounts for early fruit consumption for *V. dilatatum*.

*Implications for spread*
Herrera (1982b) posited that plants have coevolved with their dispersers to offer fruit rewards based on the dispersers seasonal needs. Where then, do invasive species fit in? Clearly, they have not had the same coevolutionary history in these new systems. However, in this study, because of differences in potential rewards there was a difference in species consuming fruit and the timing in which the consumption occurred. More importantly, how does this affect dispersal? Buckley (2006) argues that ranking fruit traits for invasibility based on dispersal is crucial for our ability to prioritize species with a higher probability of dispersal to mitigate their potential impact. This study suggests that *V. sieboldii* has a higher probability for long-distance seed dispersal because it is being consumed by migratory species. It also has shown that the bird species, Gray Catbirds and American Robins, are the primary dispersers of these invasive viburnum species. Other studies like Gleditsch et al. (2011) also found that Gray Catbird and American Robin were main consumers and dispersers of another invasive species honeysuckle (*Lonicera maackii* and *morrowii*) in Pennsylvania. Suthers et al. (2000) found that in New Jersey, Gray Catbird and American Robin only consumed fruit and were dispersers of shrub species. McCay et al. (2009) in central New York and Bartuszevife and Gorhov (2006) in Ohio, and Lafleur et al. (2007) in Connecticut have shown that generalist frugivores, especially American Robins, help spread invasive species.

Although long-distance seed dispersal is admittedly rare and hard to document, it has broad implications for spread of a plant population and population dynamics (Cain et al. 2000, Trakhtenbrot et al. 2005, Nathan et al. 2008). However, cases do exist that demonstrate long-distance dispersal. For example, Hanya (2005) showed that when frugivorous bird abundance and fruit phenology align, those plant species had higher
dispersal success. Jordano et al. (2007) demonstrated that medium-sized birds can disperse seeds a long distance, they used genetic markers to compare relatedness of fruiting parent populations to dispersed seeds to demonstrate long-distance dispersal. I believe it is also important to note that in both studies Turdus spp. were dispersers along with other birds and mammals. When comparing dispersal strategies between these two viburnum species it would seem that V. sieboldii has a distinct dispersal advantage over V. dilatatum. Despite the low probability that V. sieboldii will be dispersed a long distance it at least has a chance at long-distance dispersal where V. dilatatum does not. According to the Early Detection and Distribution Mapping System (EDDMapS) from the University of Georgia, Center for Invasive Species and Ecosystem Health, V. sieboldii (Early Detection and Distribution Mapping System 2017b) is found in twelve states while V. dilatatum (Early Detection and Distribution Mapping System 2017a) is only found in seven states. Most of these states are in the mid-Atlantic to New England but in the case of V. sieboldii there are a few mid-western states as well. However, for both species the greatest density of plants are located in New Jersey, the greater New York metropolitan area, and the greater Philadelphia area (Fig. A1). What remains to be seen is if these distributions are due to dispersal from naturalized populations or if the arboreta in these areas may act as sources for new populations. This question will be addressed in Chapter 3 when we take a landscape genetics approach to compare relatedness among populations of V. sieboldii and V. dilatatum from central New Jersey, the greater New York City area, and the greater Philadelphia area.

Over the years there have been many explanations as to why invasive species thrive in their adapted habitats, ranging from the role of propagule pressure (Lockwood et
al. 2005, Simberloff 2009), land use and disturbance (Vitousek et al. 1996), trait based comparisons (Van Kleunen et al. 2010), to competitive advantages like the enemy release hypothesis (ERH) (Williamson 1996, Keane and Crawley 2002), as well as evolutionary explanations like the evolution of increased competitive ability (EICA) (Blossey and Notzold 1995) or the evolutionary imbalance hypothesis (Fridley and Sax 2014).

However, because many different invasion theories apply to plant invasions and all invasions are context dependent (i.e. the habitat, the land use history, and the plant community composition) there are few generalizations that help to predict invasiveness or provide solutions to land managers for control and restoration efforts (Rejmánek and Richardson 1996, Goodwin et al. 1999, Alpert et al. 2000, Daehler 2003, Moles et al. 2008). Even though, invasion biologists have difficulty finding general trends of species invasions, studies like this one that focus on specific invaders in a defined region seem to have better success at determining invasion success (Davis et al. 2005, Stohlgren and Schnase 2006).

While the research about invasive species focuses on how non-natives are extraordinary at colonization and spreading, in the case of this research there may be a simpler explanation as to why at least *V. dilatatum* is a successful invader in forest understories in the northeast United States. Kominami (1987) explored if frugivorous birds remove fruit from *V. dilatatum* in their native range in Japan. What this research found was strikingly similar to what my research has shown, specifically that *V. dilatatum* fruit ripen at the beginning of fall migration, but the fruit persisted until winter and then was mainly removed by a *Turdus spp.*, in this case, Naumann’s thrush (*Turdus naumanni*). We found the exact same thing except that the *Turdus spp.* was the American
Robin. At least in this case, this suggests that invasive species, rather than having a distinct advantage over native species, are perhaps preadapted to the environment as they have the same conditions and mutualism they do in their home range. This is what allows them to be so successful. Of course, in this study we can only draw this conclusion about *V. dilatatum* as there was no literature to support that *V. sieboldii* does this in its native and introduced range but it is something to consider regarding invasive species and why it is critical to understand what species do in their native range to inform the potential of what they can do in their introduced range.

The literature suggests that these non-native viburnums may mirror niches and fruit traits of native species. For example, the native *Viburnum opulus* L. var. americanum Aiton (American cranberrybush) fruit shares many of the same traits as the non-native *V. dilatatum* fruit: potentially high sugar content, and typically isn’t consumed until the winter (Jones and Wheelwright 1987, Englund 1993, Witmer 2001). When compared to another congener native, *V. dentatum*, has a high lipid content and is consumed during fall migration (Smith et al. 2007, Smith et al. 2013) just like *V. sieboldii*. Parallel to the differences between the traits of these native species, the non-native species mirror these traits and perhaps occupy similar niches, all of which will have implications for seed dispersal. Plants have evolved these different strategies to attract birds to spread their seed and do not necessarily adhere to the conventions of native versus non-native. The benefits of the plant-frugivore interactions are dictated by the traits of plants and behavior of birds.

**Conclusion**
This study demonstrates that *Viburnum sieboldii* fruit is consumed during fall migration and is primarily dispersed by Gray Catbirds (*Dumetella carolinensis*). While *Viburnum dilatatum* fruit is consumed in both fall and winter, but mainly dispersed by American Robins (*Turdus migratorius*) in winter. Nutritional content, especially fat and energy most likely explain why *V. sieboldii* was consumed before *V. dilatatum* however, antioxidant capacity most likely explains why *V. dilatatum* was able to persist until winter because it was a deterrent for birds. The implications for spread are that *V. sieboldii* has a higher probability for long-distance seed dispersal by migratory birds in the fall whereas *V. dilatatum* is more likely to be dispersed locally by resident birds in the winter. This may explain their current distribution, but more research needs to be conducted to elucidate their spatial patterns as well as the relatedness among populations. It is also important to note that *V. dilatatum* exhibited the same fruiting phenology and mutualisms here as it does in its home range in Japan. This suggests that non-native species do not have to adapt to be successful in their introduced range but rather they are successful because a similar mutualism exists that allows the species to fulfill a similar ecological function as in their native range.

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Therneau, T. M. 2018a. Package 'coxme'.

Therneau, T. M. 2018b. Package 'survival'.


**Appendix**

Table A1. Average and standard deviation for fruit nutrition analysis for energy density (kJ/g dry mass), percent crude fat (dry mass), Folin (mg phenol GAE/g dry mass), and TEAC (µg/mg dry mass) for *Viburnum dilatatum*, *V. sieboldii* fall 2016 and *V. dentatum* fall 2015. *Standard deviation accounts for variability in trials not biological variability in fruit.*

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<th>Folin (mg phenol GAE/g dry mass)</th>
<th>TEAC (µg/mg dry mass)</th>
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<tr>
<td><em>Viburnum dilatatum</em></td>
<td>18.06±0.05</td>
<td>3.07±0.21</td>
<td>46.40±3.51</td>
<td>65.39±12.62</td>
</tr>
<tr>
<td>(Fall 2016)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Viburnum sieboldii</em></td>
<td>21.43±0.31</td>
<td>13.41±0.21</td>
<td>8.13±0.58</td>
<td>6.87±0.67</td>
</tr>
<tr>
<td>(Fall 2016)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Viburnum dentatum</em></td>
<td>28.18±0.33</td>
<td>44.53±0.59</td>
<td>55.20±3.15</td>
<td>113.55±13.45</td>
</tr>
<tr>
<td>(Fall 2015)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure A1. These are distribution maps provided by the Early Detection and Distribution Mapping System (EDDMapS) from the University of Georgia, Center for Invasive Species and Ecosystem Health for a.) Viburnum dilatatum and b.) Viburnum sieboldii.
Chapter 2: Understanding the patterns of spread: spatial distribution of two invasive ornamental viburnums on a local and statewide scale.

Abstract

Within the last decade invasive species management has shifted from controlling the spread of entrenched invasive species to early detection and eradication of newly invasive species. Understanding species distribution and abundance can help predict the potential rate of spread. Models that can reasonably detect species dispersal patterns, at both large and small scales when species populations are relatively small, are critically important. *Viburnum dilatatum* and *Viburnum sieboldii* are two relatively new invasive species whose ranges are expanding in northeastern United States forest understories. While they are not yet recognized as significant invaders, they have been noted as a local concern in New Jersey for invasiveness and the potential exists for these species to further expand their range via seed dispersal by birds. The goal of this study is to use point pattern analysis to understand the patterns of species distribution both within sites at the local community level and at statewide scales to explore potential patterns of spread. The overarching question of this study is whether the patterns of plant distribution support the observations made in chapter one about dispersal? Where *V. dilatatum* seed is dispersed locally by resident birds and *V. sieboldii* seed has the potential for long-distance dispersal because the fruit is consumed by migratory birds. I hypothesize that locally, *V. sieboldii* is more likely to spread vegetatively (clonally) once established in forest understories. In contrast, *V. dilatatum* is more likely to spread by fruit locally (gravity or bird-dispersed). Statewide (and regionally), *V. sieboldii* has a higher probability for long-distance seed dispersal because it is primarily consumed by fall migratory birds and therefore will be
found in a wider geographic area with smaller population sizes. Whereas, *V. dilatatum* is found in a smaller geographic region but with higher population density due to localized dispersal by resident birds (see Chapter 1). I found that *V. sieboldii* likely relies on clonal spread at distances under three meters but is likely bird dispersed at distances from eight to twelve meters. In contrast, *V. dilatatum* likely spreads by fruit both at close distances, under four meters by seedfall and by localized seed dispersal by birds at distances between six and thirteen meters. On the state and regional level, the patterns of distribution are less clear but will be explored more fully in the last chapter looking specifically at the relatedness between populations. However, I predict that *V. dilatatum* will have less gene flow between populations when compared to *V. sieboldii*, which likely means *V. dilatatum* is more dispersal limited.

**Introduction**

Within the last decade, invasive species management has shifted from controlling the spread of entrenched invasives to early detection and eradication of newly invasive species (Westbrooks 2004, Simberloff et al. 2013). The advantage to this approach is that both the time and the cost of managing these newly invasive species is greatly reduced compared to well-established species. There is also a higher probability that the species will be eradicated when the population is small. However, the detection and eradication of recent invaders and the ability to predict their potential distribution and methods of dispersal can be hampered by lack of information regarding the new species’ natural history as well as the ability to share information between land management agencies (Mehta et al. 2007, Simpson et al. 2009).

These difficulties demonstrate why the need to assess the potential risk of newly
introduced species is paramount to land managers, as some non-native species never
become invasive while others do. This problem is further complicated by the fact that
many invasive species can have long lag times from introduction to establishment
(Stohlgren and Schnase 2006). While invasion biologists struggle to find general trends
that describe species invasion, studies that focus on specific invaders in a defined region
seem to have better success at determining invasion success (Davis et al. 2005, Stohlgren
and Schnase 2006).

Understanding species distribution and abundance can help predict the potential
rate of spread. Models that can reasonably detect species dispersal patterns, at both large
and small scales when species populations are relatively small, are critically important.
Point pattern analysis (PPA) is a useful tool as it can be used to detect species patterns
(e.g., clumped, random, regular) at different scales. When spatial patterns are assessed,
dispersal and recruitment hypotheses can be formed to explain the underlying processes
(Wiegand and A. Moloney 2004). However, to date, only a limited number of studies
have used point pattern techniques to understand the mechanisms and processes
underlying invasion from non-native species (Call and Nilsen 2003, Deckers et al. 2005).
The majority of research using point pattern analysis has focused on explaining spatial
patterns in forest stands for use in silviculture (Reed and Burkhart 1985, Biondi et al.
Gray and He 2009). Although, there is research that uses PPA to look at recruitment
(Thorsten Wiegand et al. 2009) and spatial structure of trees (Wiegand et al. 2007), with
techniques aimed at understanding their distribution and dispersal (Wiegand and A.
Moloney 2004). Despite the fact that PPA has been underutilized for studying patterns in
invasive plant dispersal, I believe its power to explain complex ecological processes through quantitatively analyses and simulation is crucial to understanding and interpreting this inherent complexity.

As posited in the last chapter, *Viburnum sieboldii* may have the opportunity for long-distance seed dispersal because their fruit is consumed mainly by migratory birds, while *Viburnum dilatatum* seed dispersal may be limited because primarily resident birds are consuming the fruit. If this is the case, the patterns of spread should be evident at a regional scale with *V. sieboldii* having populations spread over a wider geographic area while populations of *V. dilatatum* are more closely clumped. On a local scale, the community composition may be the inverse of the regional, with *V. sieboldii* found in clumps due to single dispersal events from a migratory bird and then clonal spread once established, while *V. dilatatum* would demonstrate regular (hyperdispersed) patterns of dispersal as a result of resident birds spreading its seed.

The goal of this chapter is to determine patterns of species distribution both within sites, to understand local community patterns of spread and at a statewide scale, to explore potential patterns of spread (as reliable regional data is not available). For determining statewide patterns, detection data from the New Jersey Invasive Species Strike Team (NJISST) will be used for both viburnum species. For determining local distribution patterns, macroplots will be established for each viburnum species. In each macroplot, the location of individuals will be mapped and basal diameter measured for use in point pattern analysis. The overarching question of this study is whether the patterns of plant distribution support the observations made in chapter one about dispersal? Where *V. dilatatum* seed is dispersed locally by resident birds and *V. sieboldii
seed has the potential for long-distance dispersal because the fruit is consumed by migratory birds. I hypothesize that locally, \textit{V. sieboldii} is more likely to spread vegetatively (clonally) once established in forest understories \{personal observation\}. In contrast, \textit{V. dilatatum} is more likely to spread by fruit locally (gravity or bird-dispersed). Statewide (and regionally), \textit{V. sieboldii} has a higher probability for long-distance seed dispersal because it is primarily consumed by fall migratory birds and therefore will be found in a wider geographic area with smaller populations size. In contrast, \textit{V. dilatatum} is likely found in a smaller geographic region but with higher population density due to localized dispersal by resident birds. This research hopes to support the hypothesis put forth in the first chapter by demonstrating that the patterns of propagule strategies for each viburnum species, locally and statewide. By exploring spatial distribution patterns, we can more fully explore if indeed the differences in the types of dispersal result in differences in plants distributions. This information could be used to inform predictive models for species distributions.

**Methods**

\textit{Species distribution and life history}

Both viburnum species were brought over from East Asia as ornamental specimens in the mid to late 1800s. They were valued because of their showy white flowers and attractive fruit as well as their adaptability to a wide range of climatic and soil conditions (Rehder 1927, Dirr 1990). They are both are understory species however, they differ in growth form. \textit{Viburnum dilatatum} is a is multi-stemmed shrub that can reach 3 meters in height and 2 meters in canopy width when fully mature. While \textit{Viburnum sieboldii} is generally a single-trunked, subcanopy tree that can reach 6 meters
in height and 4.5 meters in canopy width when fully mature (Dirr 1990).

Within the past thirty years both species have been expanding their range in the Northeast and some Midwest States (Early Detection and Distribution Mapping System 2017b, a). Currently, *V. dilatatum* (Early Detection and Distribution Mapping System 2017a) is found in seven states and *V. sieboldii* (Early Detection and Distribution Mapping System 2017b) is found in twelve states according to the national map from EDDMapS (Fig. 2). While they are not yet recognized as significant invaders, they have been noted as a local concern for invasiveness in New Jersey, New York, Pennsylvania, and Maryland (Glenn and Moore 2010, Martin and Burgiel 2012, Pennsylvania Department of Conservation and Natural Resources 2012a, b, New Jersey Invasive Species Strike Team 2017) because they displace native species.

**Study area and sampling plots**

This study examines dispersal of *Viburnum dilatatum* and *Viburnum sieboldii* on two scales: a statewide level and a local level. New Jersey is the focal state and Lewis Morris County Park (park main entrance-UTM Zone 18T, 539460.76 m E, 4515753.78 m N) located in Morristown, in Morris County, NJ (Fig. 1) is the focal site for local level population dispersion.
New Jersey was selected for this study because it has the highest density of both viburnum species according to the Early Detection and Distribution Mapping System (EDDMapS) from the University of Georgia, Center for Invasive Species and Ecosystem Health (Early Detection and Distribution Mapping System 2017a, b) (Fig. 2). It was also selected because it is the only state in the EDDMapS system that has point locations of populations reported by the New Jersey Invasive Species Strike Team (NJISST). Lewis Morris County Park was selected for this study because both viburnum species have naturalized and well-established populations in this park.
Figure 2. These distribution maps from the Early Detection and Distribution Mapping System (EDDMapS) from the University of Georgia, Center for Invasive Species and Ecosystem Health for a) *Viburnum dilatatum* and b) *Viburnum sieboldii*.

**New Jersey State Data**

Data for New Jersey populations of *Viburnum dilatatum* and *Viburnum sieboldii* was downloaded from the New Jersey Invasive Species Strike Team (NJISST) website on April 25, 2017 (New Jersey Invasive Species Strike Team 2017). The data included a shapefile with point locations reported in decimal degrees in the coordinate reference system (CRS) WGS 84 (EPSG:4326) and a Microsoft Excel spreadsheet containing collection notes with supplementary material (e.g., date reported, property name, land manager, habitat type, treatment status, number of individuals within a population). Data collection started in 2008 by the Central Jersey Invasive Species Strike Team and was self-reported by volunteers. In 2011, the program expanded statewide as the NJISST which resulted in invasive species monitoring efforts by NJISST as well as partnerships between private and public organizations across all levels of government. In 2014, the efforts were expanded further by Charles T. Bargeron from the University of Georgia Extension, Center for Invasive Species and Ecosystem Health by developing an NJISST app that allows citizens to report invasive species that are then verified by the NJISST
pers. comm. Mike Van Clef (New Jersey Invasive Species Strike Team 2017). This dataset includes nearly nine years of reporting locations for both viburnum species (Fig. 3).

NJISST uses two categories to gauge the species impact: emerging stage (0-3 or widespread) which is based off of the number of detections to determine statewide distribution, as well as a threat level (mild-moderate-high) to assess a species potential to harm natural areas. While NJISST considers both viburnum species to be a high threat to natural areas due to invasion potential, *V. dilatatum* is considered widespread and common in New Jersey because there are more than 1,000 detections reported and the species is believed to be causing significant harm in natural areas by displacing native species. While *V. sieboldii* is at an emerging stage 2, meaning it is uncommon but may be abundant regionally because there are only between 101-500 detections reported (New Jersey Invasive Species Strike Team 2017).

![Maps](image)

Figure 3. Population locations for New Jersey state data for a) both *Viburnum dilatatum* and *Viburnum sieboldii*, b) *Viburnum dilatatum* NJ state data represented by red dots and c) *Viburnum
Data for local level dispersal was collected at Lewis Morris County Park. An exhaustive survey was conducted within the park during summer 2016 to locate viburnum populations and identify potential plots. Criteria for plot selection was based on the highest total number of individuals per population; potential plots needed to have a baseline population consisting of more than one hundred individuals to be considered for selection. After completion of the survey, two locations were selected; one location per viburnum species. At these locations 50X50 meter macroplots were set up by establishing a southeast corner and marking it with a wooden stake. From that stake two 50 m tape measures (Keson Industries, Aurora, Illinois, USA) were laid out orthogonally from the southeast corner to establish the southwest and northeast corners, again marked by wooden stakes. Another 50 m tape was then extended westward from the northeast corner to establish the northwest corner, which was marked by a wooden stake. Finally, a fourth tape was extended from the northwest corner to the southwest corner and corrections to the plot were made as necessary if the plot boundary was not a perfect square. Once the plots were established the boundaries were marked with lime-green flagging tape and the corners were geo-referenced with a Trimble GeosXH with Tornado antenna (Trimble Navigation Limited, Sunnyvale, California, USA) in UTM Zone 18T. The centroid point for *Viburnum dilatatum* macroplot is 539089 m Easting, 4514898 m Northing and centroid point for *Viburnum sieboldii* macroplot is 538894 m Easting, 4514572 m Northing (Fig. 4). During fall 2016, all individuals that fell within the plots were marked by a unique identification in the form of numbered aluminum tags. In winter 2017, all individuals were geo-referenced with the Trimble GeosXH with Tornado antenna (UTM
Zone 18N). Geo-referencing was done in the winter to ensure that leaf canopy cover was completely gone which improved the overall accuracy of the Trimble. Accuracy for points collected need to be less than 1.3m to be accepted which generally meant accumulating twenty-five to fifty replications per point with four or more satellites for improved accuracy. All individuals in the plot were measured for basal diameter from the largest shoot on each individual ten centimeters up from base of plant and recorded on a datasheet with corresponding identification number. If the shoot was equal to or less than one centimeter it was measured with dial calipers (SPI, Garden Grove, California, USA) and if the shoot was greater than one centimeter it was measured with 5 m diameter tape (Forestry Suppliers Inc, Jackson, Mississippi, USA).
Figure 4. Locations of 50x50 meter macroplots within Lewis Morris County Park a) Topography map showing the elevation of each plot as well as where they are in relation to each other b) Satellite map showing forest cover of plots c) Viburnum dilatatum macroplot and d) Viburnum sieboldii macroplot with individual shrubs represented by white dots.

**Data processing and analysis**

All spatial data from the Trimble GeosXH was downloaded via GPS Pathfinder Office. All raw macroplot data was compiled into an excel file; all curated excel files and shapefiles for spatial data for macroplots and NJ State data was loaded, processed and analyzed in RStudio (RStudio Team 2016) and any overlapping point data was removed. All shapefiles were opened and edited in QGIS (Quantum GIS Development Team 2017, Quantum GIS Geographic Information System, Open Source Geospatial Foundation)
Project, [https://qgis.org/en/site/](https://qgis.org/en/site/) for use in analysis in RStudio or to create maps. For NJ state data, the coordinate system was projected in CRS WGS 84 (EPSG:4326) and for macroplot data, the coordinate system was projected in CRS WGS 84/UTM Zone 18T (EPSG:32618). Figures were edited or modified in Inkscape (Harrington, B. et al 2004-2005, Inkscape, [https://inkscape.org/en/](https://inkscape.org/en/)). Base layer for the state of New Jersey and its counties was provided by The State of New Jersey, Department of Environmental Protection, Bureau of GIS and boundary maps provided by Janice Karmon, GIS Coordinator, Park Planning & Development for the Morris County Park Commission.

**Models and equations**

NJ state data was treated qualitatively because of collection bias over the nine-year span of data collection. This bias resulted because the data was collected by multiple organizations and volunteers which led to changes in sampling protocol and an inconsistent sampling effort over the nine-year span. Also, population estimates for each point have varying ranges from 1, 2-10, 11-100, and <100 individuals, which precludes any point pattern analysis as the population wasn’t exhaustively sampled. Plus, each point has varying stages of pesticide treatment (no action, initiated, eradicated) so it would be hard to know if it is the same population is regrowing or it is a genuinely new population. Therefore, the NJ point data was assessed by a density function with and without a Diggle’s correction (Diggle 1985, Berman and Diggle 1989, Diggle 2003, Baddeley et al. 2015) that smooths the bandwidth for the estimation of the kernel of the point pattern by minimizing the mean-square error. This function displays a heat map for the number of points per unit area.
Macroplot data was treated quantitatively with point pattern analysis (PPA) which is used to analyze the spatial patterns within the macroplots to determine dispersion patterns that can be used to help inform the underlying ecological processes affecting dispersal of each viburnum species. Three types of summary statistics were used to determine point pattern analysis, pair correlation function (PCF) $g(r)$, mark correlation $k_{mm}(r)$, and mark variogram $\gamma_{m}(r)$. Since the macroplot data is a univariate analysis (individuals of a single species) a PCF will compare the macroplot data to a null model of complete spatial randomness (CSR). This model will help determine if the points within the macroplot are dispersed, random, or clumped at given distances. This will provide insight into the underlying dispersal mechanisms for each species within the plot. The underlying assumption for this analysis is that the points are homogeneous, meaning that underlying environmental conditions and processes are the same everywhere within the observable window (Illian et al. 2008, Wiegand and Moloney 2014, Baddeley et al. 2015). However, data was analyzed as homogeneous and inhomogeneous for the PCF. The spatial homogeneity assumption was tested by dividing the plot up into quadrats and performing a chi-squared test to see if observed patterns deviate from expected model of CSR in order to select the appropriate interpretation of the PCF. The PCF equation for homogeneous is as follows

$$g(r) = K'(r)/2\pi r$$  \hspace{1cm} (1)

where $g(r)$ is equal to the derivative of $K'(r)$ with respect to $2\pi r$ which is the distance over the area of the ring (Illian et al. 2008, Wiegand and Moloney 2014, Baddeley et al. 2015). The PCF equation for inhomogeneous is as follows

$$g_{inhom}(r) = K'_{inhom}(r)/2\pi r$$  \hspace{1cm} (2)
and considers that the dependence between points does not have a uniform density of points with the following equation

\[ p(r) = \lambda(x) \lambda(y) g(r) \, dx \, dy \]  

where \( p(r) \) is the probability of finding two points at locations \( x \) and \( y \) separated by distance \( r \) and where \( \lambda \) is the intensity function of the point process (Baddeley et al. 2015).

Since PCF measures spatial association in rings and not cumulative circles like Ripley’s K function, it will show a pattern of points at any given scale which is useful when determining patterns of dispersal. If \( g(r) > 1 \) it means that points are more clustered than expected under CSR. If \( g(r) < 1 \) it means points are more dispersed than expected under CSR and \( g(r) = 1 \) random dispersal (Illian et al. 2008, Szymt 2014, Wiegand and Moloney 2014, Baddeley et al. 2015). The pair correlation function ran ninety-nine simulated realizations of the model for use in a Monte Carlo test and will display simulation envelopes. PCF estimates that falls outside of the envelope are considered to be statistically different from random.

A mark correlation analysis was performed to determine if there is dependence between two marked points at a given distance (Illian et al. 2008, Wiegand and Moloney 2014, Baddeley et al. 2015). In other words, it will take into account how the mark, which in this case is the basal diameter of the individual viburnum, influences the spatial patterns observed. The test function for mark correlation (equation 3.88 from Wiegand and Moloney 2014) equation is as follows

\[ k_{mm}(r): t_1(m_i, m_j) = m_i \, m_j \]
where \( k_{mm}(r) \) is the mark correlation, \( m_i \) and \( m_j \) are the marks of two points \( i \) and \( j \). So, if \( k_{mm}(r)>1 \) it means that points located at a given distance \( r \), have a larger mean mark (stimulation) or if \( k_{mm}(r)<1 \) points at a given distance \( r \), have a smaller mean mark (inhibition). \( k_{mm}(r)=1 \) means no correlation (Illian et al. 2008, Szmyt 2014, Wiegand and Moloney 2014, Baddeley et al. 2015). The test function is derived from the mark correlation equation, 3.84 and 3.85 in Wiegand and Moloney (2014) and the spatial correlation between marks is normalized by equation 3.87 and the expectations are found in equation 3.89. The mark correlation test function ran ninety-nine simulated realizations of the model for use in a Monte Carlo test and will display simulation envelopes. Data that falls outside of envelope is different than expected.

As a complement to the mark correlation analysis, a mark variogram analysis was also performed. The mark variogram is a measure of the dependence between the marks of point pairs as a function of distance, essentially determining if individual’s marks are similar or different than its neighbor at a given distance \( r \) (Illian et al. 2008, Wiegand and Moloney 2014, Baddeley et al. 2015). The mark variogram has small values if the marks of the points are similar in magnitude and large values if the marks of the points are different (Illian et al. 2008, Szmyt 2014, Wiegand and Moloney 2014, Baddeley et al. 2015). The test function for the mark variogram (equation 3.88 from Wiegand and Moloney 2014) equation is as follows

\[
\gamma_m(r): \tau_d(m_i, m_j) = (m_i - m_j)^2/2
\]  

(5)

where \( \gamma_m(r) \) is the mark variogram, \( m_i \) and \( m_j \) are the marks of two points \( i \) and \( j \) and squares the difference and divides by a half (Illian et al. 2008, Szmyt 2014, Wiegand and Moloney 2014, Baddeley et al. 2015). Again, the test function is derived from the mark
correlation equation, 3.84 and 3.85 in Wiegand and Moloney (2014) and the spatial correlation between marks is normalized by equation 3.87 and the expectations are found in equation 3.89. The mark variogram function ran ninety-nine simulated realizations of the model for use in a Monte Carlo test and will display simulation envelopes. Data that falls outside of envelope is different than expected.

Macroplot data was used to generate plot point maps to show individual plants location within the macroplot as well as heat maps (with and without a Diggle correction) to represent the number of individuals per unit area. Macroplot mark data was used to create basal diameter classes as well as density functions for the mean and variance of the basal diameter of individuals within the plots and accompanying histograms. All figures for viburnum NJ state data, including plot point data and plot density were created in RStudio. For viburnum macroplot data, all figures and analyses for PCF, mark correlation, and mark variogram were created in RStudio using the package spatstat (Baddeley et al. 2015). Additionally, figures showing plot point data, plot density maps, histograms for viburnum basal diameter were also created in RStudio.

Results

New Jersey State Data

NJISST reported 1395 data points for Viburnum dilatatum in New Jersey, 1388 of which were unique points (Fig. 5a). The density map (Fig. 5b) shows the highest density per unit area at the

![Figure 6. Map of New Jersey counties with associated table for population numbers per county for Viburnum dilatatum and Viburnum sieboldii.](image-url)
western edge of Mercer County extending and decreasing in density, northeast towards Morris County but also touching parts of Monmouth County. Diggle’s correction (Fig. 5c) dramatically decreases the area of the hotspots to localized populations with many populations found in Mercer, Morris, and the greatest density found in Monmouth County. *V. dilatatum* was found in 9 counties in New Jersey with Mercer County being the highest number of populations at 653, followed by Monmouth at 165, Somerset at 146, Union at 137, Morris at 134, Hunterdon at 82, Essex at 42, Middlesex at 27, and Warren at 2 populations of *V. dilatatum* (Fig. 6).

**Viburnum dilatatum** point and density maps

![Figure 5. Three different maps of *Viburnum dilatatum* populations within New Jersey. a) Locations of 1388 unique points for *Viburnum dilatatum* populations within New Jersey, b) density map showing the majority of populations fall within Mercer County, NJ and radiate northeast, and c) density map with Diggle’s correction dramatically decreases the area of hotspots to localized populations.](image)

NJISST reported 344 data points for *Viburnum sieboldii* in New Jersey, 339 of which were unique points (Fig. 7a). The density map (Fig. 7b) shows the highest density per unit area at the western edge of Mercer County extending and decreasing in density northeast towards Morris County. Diggle’s correction (Fig. 7c) dramatically decreases the area of the hotspots to localized populations with many populations found in Mercer and the greatest density found in Morris County. *V. sieboldii* was found in 8 counties in New
Jersey with Mercer County being the highest number of populations at 161, followed by Morris at 117, Somerset at 27, Essex at 26, Middlesex at 3, Monmouth and Union at 2, and Hunterdon at 1 for *V. sieboldii* (Fig. 6).

**Viburnum sieboldii** point and density maps

Figure 7. Three different maps of *Viburnum sieboldii* populations within New Jersey. a) Locations of 339 unique points for *Viburnum sieboldii* populations within New Jersey, b) density map showing the majority of populations fall within Mercer County, NJ and move linearly northeast, and c) density map with Diggle’s correction dramatically decreases the area of hotspots to localized populations.

**Macroplots**

There was a total of 461 individuals within the *Viburnum dilatatum* 50X50 m macroplot (Fig. 8a), ranging in basal diameter from 0.5 to 5.3 cm. The density map (Fig. 8b) shows the highest density per unit area at the northernmost end of the plot and decreasing in density towards the southeast corner with the average intensity being 0.1844 points per square meter. The density map with the Diggle’s correction (Fig. 8c) provides a more nuanced representation with the highest density of individuals still represented in the north of the plot but patches of high density surrounding that northernmost patch with very low density in the southeast corner of the plot.
There was a total of 349 individuals within the *Viburnum sieboldii* 50X50 meter macroplot (Fig. 8a), ranging in basal diameter from 0.8 to 18.8 cm. The density map (Fig. 8b) shows the highest density per unit area at the southernmost end of the plot, extending and decreasing in density towards the northern end of the plot with the average intensity being 0.1396 points per square meter. The density map with the Diggle’s correction (Fig. 8c) provides a more nuanced representation with the highest density of individuals still represented in the south end of the plot but arms of high density extending north, east and west but with very low density in the northeast corner of the plot.

*V. dilatatum* basal diameter ranged from 0.5 to 5.3 cm with the cutoff for the 1st
quartile at 1.7 cm and for the 3rd quartile at 2.8 cm (Fig. 10c). The mean basal diameter was 2.3 cm. The distribution of basal diameter for *V. dilatatum* conformed to a normal distribution with a Shapiro-Wilk normality test of a W of 0.98864 and a p-value of 0.001203 (Fig. 10a). *V. sieboldii* basal diameter ranged from 0.8 to 18.8 cm with the cutoff for the 1st quartile at 3.3 cm and for the 3rd quartile at 8.0 cm. The mean basal diameter being 5.1 cm (Fig. 10c). The distribution of basal diameter for *V. sieboldii* conformed to a normal distribution with a Shapiro-Wilk normality test of a W of 0.95465 and a p-value of 6.701e-09 (Fig. 10b).
Figure 10. Basal diameters of viburnum individuals within macroplot. a) Basal diameter distribution for *Viburnum dilatatum* and b) basal diameter distribution for *Viburnum sieboldii*. c) How the basal diameters compare between the two species of viburnum with *Viburnum sieboldii* individuals are larger, on average, than *Viburnum dilatatum* individuals.

**Pair correlation**

The pair correlation for *Viburnum dilatatum* with the assumption of spatial homogeneity showed that *V. dilatatum* individuals exhibit clustering at all distances but
most significantly at distances from approximately zero to four meters. Distances greater than four meters are still significantly different however, they are closer to the simulation envelopes compared to distances between zero to four meters (Fig. 11a). However, the pair correlation for *V. dilatatum* with the assumption of spatial inhomogeneity showed that *V. dilatatum* individuals are only clustered at distances from approximately one to four meters and then taper off into the simulation envelope between four and six meters meaning at this distance they are randomly spaced before emerging below the bottom border of the simulation envelope suggesting a hyper-dispersed spacing at distances from six to thirteen meters (Fig. 11b).

![Figure 11. Pair correlation of *Viburnum dilatatum* individuals within macroplot with underlying assumption of a) spatial homogeneity and b) inhomogeneity.](image)

The pair correlation for *Viburnum sieboldii* with the assumption of spatial homogeneity showed that *V. sieboldii* individuals exhibit clustering at all distances but most significantly at distances from approximately one to five meters. Distances greater than five meters are significantly different than the simulation window however, they are closer to the simulation envelopes compared to between one to five meters (Fig. 12a). However, the pair correlation for *V. sieboldii* with the assumption of spatial inhomogeneity showed that *V. sieboldii* individuals are only clustered at distances from
approximately one to three meters and then taper off into the simulation envelope
between three and eight meters meaning at this distance they are randomly spaced before
emerging below the bottom border of the simulation envelope between eight and twelve
meters suggesting dispersed spacing at those distances (Fig. 12b).

The chi-squared test for CSR for both species showed that each species’ patterns
within the macroplot were inhomogeneous. *V. dilatatum* was divided into 25 windows
with irregularity (meaning all quadrats were not complete squares) and had a $X^2$ of
125.02, with degrees of freedom of 24, and a $p$-value of 2.453e-15. *V. sieboldii* was
divided into 21 irregular windows with a $X^2$ of 105.81, degrees of freedom of 20, and a $p$-
value of 2.267e-13. Therefore, the pair correlations with the inhomogeneous assumptions
are the more accurate interpretations.

**Mark correlation**

The mark correlation for *Viburnum dilatatum* individuals suggests that basal
diameter of individuals is independent or uncorrelated at all distances with values near
one and within the simulation envelopes (Fig. 13a). The mark variogram for *V. dilatatum*
shows that individuals are generally similar in size to their nearest neighbor however, the
data never deviates from the simulation envelopes significantly (Fig. 13b).

Figure 13. Taking into account how basal diameter of *Viburnum dilatatum* individuals influence spatial patterns within the macroplot. a) Mark correlation tells us if basal diameter is stimulated, inhibited, or independent by its nearest neighbor at given distances and b) mark variogram infers if points basal diameters are more similar or less similar at given distances.

The mean for basal diameter for *V. dilatatum* individuals within the macroplot was 2.3 cm and figure 14b shows a fairly even distribution of all basal diameters throughout the plot. The variance of the basal diameter for *V. dilatatum* was on average 0.5 cm for the plot and figure 14c shows the greatest variance seems to concur with areas that have individuals with a larger basal diameter.

Figure 14. Three different maps of *Viburnum dilatatum* individuals within 50X50 m macroplot. a) Locations and basal diameter of 461 *Viburnum dilatatum* individuals within the plot, b) density map of basal diameters of individuals spread evenly throughout the plot and c) the density map of basal diameter variance shows the greatest variance around pockets of large basal diameter individuals.

The mark correlation analysis for *Viburnum sieboldii* individuals suggests that basal diameter of individuals is significantly larger than the average basal diameter at
distances from two meters and beyond, meaning stimulation. This can also be interpreted to mean that large trees are associating with large trees and small trees associate with small trees. Less than two meters from the nearest neighbor size relationships tend to be random (uncorrelated) as the data stays within the simulation envelopes (Fig. 15a). The mark variogram for *V. sieboldii* shows that individuals are generally similar in size to their nearest neighbor especially at distances approximately from zero to five meters, then data falls within the simulation envelopes (Fig. 15b).

Figure 15. Taking into account how basal diameter of *Viburnum sieboldii* individuals influence spatial patterns within the macroplot. a) Mark correlation tells us if basal diameter is stimulated, inhibited, or independent by its nearest neighbor at given distances and b) mark variogram infers if points basal diameters are more similar or less similar at given distances.

The mean basal diameter for *V. sieboldii* individuals within the macroplot was 5.3 cm and figure 16b shows individuals with larger basal diameters found towards the south and center of plot. The variance of the basal diameter for *V. sieboldii* was on average 6.8 cm for the plot and figure 16c shows that the greatest variance seems to occur within the center of the plot which also has the largest basal diameter individuals.
Figure 16. Three different maps of *Viburnum sieboldii* individuals within 50X50 m macroplot. a) Locations and basal diameter of 349 *Viburnum sieboldii* individuals within the plot, b) density map of basal diameters of individuals are clumped near the south and center of the plot and c) the density map of basal diameter variance shows the greatest variance around large basal diameter individuals.

**Discussion**

**New Jersey State Data**

There are four times more *Viburnum dilatatum* populations than populations of *Viburnum sieboldii* in New Jersey. However, the population density for both species are relegated to northern New Jersey. Based on solely on the number of detections and population density (Fig. 5 and 6), *V. dilatatum* is the more successful invader in New Jersey compared to *V. sieboldii*. However, if we consider the national map from EDDMapS in figure 2 we see that *V. sieboldii* (Early Detection and Distribution Mapping System 2017b) is found in a total of thirty-eight counties spread over twelve states while *V. dilatatum* (Early Detection and Distribution Mapping System 2017a) is found in thirty-seven counties but only found in seven states. Based off this map (Fig. 2) we can also see that *V. dilatatum* is limited to the mid-Atlantic where it has the highest densities in New Jersey, New York, parts of Pennsylvania and counties surrounding Washington, D.C. On the other hand, *V. sieboldii* is also found in the mid-Atlantic with the highest densities in New Jersey, New York, and eastern Pennsylvania, but can also be found as far north as
Massachusetts and as far west as Illinois. Therefore, *V. sieboldii* is more successful invader on a regional scale.

The distributions shown on the maps from EDDMapS are a combination of verified observations from federal, state, and local governments, non-profit organizations, and volunteers, state flora atlas databases, state flora checklists, as well as specimens from arboreta herbariums. Figure A1 gives a breakdown of what reports were observations and what came from the literature (e.g. state flora atlas databases, state flora checklists, as well as specimens from arboreta herbariums). Understanding when and where these species have been observed can shed some light on whether these naturalized populations have spread from other naturalized populations or from areas where they were cultivated in arboreta and botanical gardens.

EDDMapS was created in 2005 so the bulk of all data for both species ranges from 2005-2017. However, this does not give us a sense of when these species were first reported as naturalized in each state. The earliest record of a naturalized *V. dilatatum* population in the U. S. is for Pelham Bay Park in the Bronx in a 1946-1947 survey by Ahles (DeCandido and Lamont 2004). There is also a 1951 record for *V. dilatatum* in Montgomery County in eastern Pennsylvania (Snyder 1987). Other records for *V. dilatatum* populations in the mid-Atlantic region show up through the 1970s through the 1990s in New Jersey, Pennsylvania and New York (Greller et al. 1982, Mitchell 1986, Snyder 1987, Greller 1989, DeCandido and Lamont 2004). However, the majority of records in the region begin in the 2000s and include populations in Connecticut, Delaware, and the Washington D.C. metropolitan area, as well as new populations in New York (McAvoy and Bennett 2001, Torrey Botanical Society 2005, Steury et al. 2008,
Torrey Botanical Society 2008, Morgan 2009, Dean et al. 2011, Steury 2011). Outside of the mid-Atlantic and not included in EDDMapS records are populations in Kentucky (Gunn 1959) and Illinois (Basinger 1999). The increase in reported naturalized *V. dilatatum* populations was cause enough for concern that between 2010-2012 at least four organizations in four states recognized the potential threat to natural communities {2010, NJISST, New Jersey; 2010 Partnership for Regional Invasive Species Management (PRISM), New York; 2012, Department of Conservation and Natural Resources, Pennsylvania; 2012 The National Park Service, National Capital Region’s Exotic Plant Management Team, Washington D.C. metropolitan area (Glenn and Moore 2010, Dean et al. 2011, Martin and Burgiel 2012, Pennsylvania Department of Conservation and Natural Resources 2012a, New Jersey Invasive Species Strike Team 2017)}. The earliest record of naturalized *V. sieboldii* populations in the U. S. are from 1979 in Bristol, Hampshire, and Worcester counties in Massachusetts (Sorrie and Somers 1999, Sorrie 2005). Only one record exists earlier, from 1976, in Amherst, Massachusetts (Hampshire county) although it is not clear if it was a cultivated or a naturalized individual (Jennings and Timmerman 1976). Throughout the 1990s populations of *V. sieboldii* were found in New Jersey, New York, Pennsylvania, Ohio, and Kentucky, although it is not always clear from the records whether the populations should be considered cultivated or naturalized (Yost et al. 1991, Künstler 1993, Rhoads and Klein Jr. 1993, Weckman et al. 2002, Vincent et al. 2011, Morristown National Historical Park 2015). Naturalized populations in Connecticut, Massachusetts, Maryland, Washington D.C., Delaware, Virginia, West Virginia, and Illinois were found in the 2000s, along with additional populations in New York and Ohio (McAvoy and Bennett 2001, Weckman et

Reports from the literature note when these species were found as naturalized and they also sometimes mention the potential cultivated source for these naturalized populations. For example, in the Washington D.C. area Steury (2011) believes the naturalized populations of *V. dilatatum* are from historical plantings along the George Washington Memorial Parkway, noted in plans developed by Wilbur Simonson in 1932. In Illinois, Basinger believes that *V. dilatatum* became naturalized because of original plantings at the Marberry Arboretum in Carbondale. In Connecticut, Morgan points to *V. dilatatum* use in ornamental plantings throughout the state but does not give specific details as to where or when they were planted. In New York, Yost (1991) posits that *V. sieboldii* established in Wave Hill Natural Area in the Bronx do to the combination of disturbance from land use and ornamental plantings in the vicinity allowed non-natives to take hold. However, Künstler notes that *V. sieboldii* likely naturalized in Wave Hill because it was cultivated in the gardens there (Künstler 1993). The same is true in Connecticut at Bartlett Arboretum where Morgan believes *V. sieboldii* escape from the
cultivated specimens on the grounds. In Washington D.C. there are specimens of *V. sieboldii* in the herbarium from the Smithsonian Institution from the U.S. National Arboretum as early as 1907 and in Maryland there is a specimen in the Norton-Brown Herbarium at the University of Maryland that dates back to 1958. In Massachusetts, both Wyman and Egolf (Wyman 1937, Egolf 1956) mention that *V. dilatatum* and *V. sieboldii* in the collection of the Arnold Arboretum in Boston. In Rehder’s Manual of Cultivated Trees and Shrubs (1927) states that *V. dilatatum* has been in cultivation since 1919 and *V. sieboldii* since 1880.

Indeed, it is not uncommon that both species are in arboreta and botanical gardens collections. While visiting arboreta and botanical gardens in New York, New Jersey, and Pennsylvania scouting for research locations I noted which species where found in the collections (Table 1). While by no means is this an exhaustive search for these two species it illustrates the larger point that in many cases these two viburnums were already cultivated in many states and it seems likely that they could have spread from there. There could be many explanations for the naturalized populations distribution that could be due to the vectors and timing of dispersal or if they were controlled early on in one state and largely ignored or undetected in another state. Clearly, there seems little doubt that historical ornamental plantings have helped propel both species spread through dispersal.

For example, in chapter one it was shown that *V. sieboldii* fruit is dispersed during fall migration, mainly by Gray Catbirds (*Dumetella carolinensis*) and that *V. dilatatum* fruit is dispersed in the winter, mainly by American Robins (*Turdus migratorius*). The implications for spread are that *V. sieboldii* have a higher probability for long-distance
seed dispersal by migratory birds in the fall whereas *V. dilatatum* are more likely to be dispersed locally by resident birds in the winter. Certainly, these patterns seem to be borne out on the national map and even at a state level we see that *V. dilatatum* is packed in a smaller geographic area with higher densities. This would be expected if the seed does not travel far from the parent plant, as you would have many individuals in a smaller geographic area. Although *V. sieboldii* also has high densities in New Jersey as well, it also has established populations in a wider geographic area. However, to understand regional patterns it is useful to look at localized patterns of dispersal.

Table 1. Research locations where one or both viburnum species was used in plantings or as a part of a collection in an arboretum or botanical garden. * Because of improper record keeping the approximate year of planting is unknown however, both *Viburnum* are large specimens.
## Viburnum found in arboreta and botanical gardens collections

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Arboretum/Botanical Garden</th>
<th>Species</th>
<th>Year(s)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>Montgomery</td>
<td>Barnes Arboretum</td>
<td>Viburnum sieboldii</td>
<td>1970s</td>
<td>William Reim, Living Collections Associate and Horticulture Programs Coordinator, Barnes Arboretum</td>
</tr>
<tr>
<td></td>
<td>Montgomery</td>
<td>Barnes Arboretum</td>
<td>Viburnum sieboldii</td>
<td>1942</td>
<td>Anthony Axtillo, The Gayle E. Maloney Director of Horticulture and Curator, Morris Arboretum</td>
</tr>
<tr>
<td></td>
<td>Philadelphia</td>
<td>Morris Arboretum</td>
<td>Viburnum sieboldii</td>
<td>1943</td>
<td>Anthony Axtillo, The Gayle E. Maloney Director of Horticulture and Curator, Morris Arboretum</td>
</tr>
</tbody>
</table>

## Macrosplots
There are important differences to note between the two species and their macroplots as it may help to explain spatial patterns within the plots. First, *V. dilatatum* has over 122 more individuals per plot than *V. sieboldii* and, on average, *V. sieboldii* basal diameter is greater than that of *V. dilatatum* (Fig. 10c). The differences in basal diameter can be attributed to the fact that *V. sieboldii* is generally a single-trunked, subcanopy tree that can reach 6 meters in height and 4.5 meters in canopy width when fully mature. While *V. dilatatum* is multi-stemmed shrub that can reach 3 meters in height and 2 meters in canopy width when fully mature (Dirr 1990). These differences in growth form and density of shrubs per plot between the two species may affect the patterns of each individual’s establishment within a plot.

There are apparent differences when comparing the density plots between the two species that are supported by interpretation of the results from the pair correlation and mark correlation functions. However, there is one commonality that both species share between plots which is neither are homogeneous and therefore the inhomogeneous data should be used for interpretations as it is a more accurate representation of the underlying ecological processes. We can see this in the density map for both species and it was verified by running a chi-squared test on the distribution of individuals within the macroplot. The *V. dilatatum* macroplot has a high density in the northernmost part of the plot and very low in the southeast corner (Fig 17a). *V. sieboldii*, patterns are also not spatially homogeneous as densities cluster at the southern end of the macroplot and extend in a peninsula of high density toward the north end of the plot (Fig 17d).

The inhomogeneous pair correlation for *V. dilatatum* individuals (Fig. 11b) supports what the density map shows (Fig. 17a) that *V. dilatatum* individuals are only
significantly clustered at distances of approximately one to four meters. Between four and six meters *V. dilatatum* individuals are randomly spaced. At distances from six to thirteen meters *V. dilatatum* exhibits dispersed spacing and then random spacing at distances greater than thirteen meters. The mark correlation lends insight into how basal diameter affects the relationships among the individual *V. dilatatum* shrubs. However, it seems that for *V. dilatatum* individuals, basal diameter of individuals is uncorrelated at any distance (Fig. 13a). The mark variogram also suggests that individuals are generally similar in size to their nearest neighbor although not significantly so, as the data never deviates from the simulation envelopes (Fig. 13b). Figure 17b and 17c supports the mark correlation and variogram, respectively. Figure 17b shows that mean mark is fairly evenly distributed for most of the plot and where variance is highest is also around the higher than average basal diameter. This suggest that different size classes are evenly dispersed throughout the plot. When breaking basal diameter into size class by their quartiles, this trend becomes apparent. Figure 18 shows late, mid-, or early classes based on their size, the assumption being that the larger the basal diameter, the older the individual. Where there are late or older shrubs (Fig. 18a), there are also mid- (Fig. 18b), and early or young shrubs (Fig. 18c) this especially stands out when all the points are overlaid (Fig. 18d).
Figure 17. The lay of the land. a) Density of *Viburnum dilatatum* individuals, b) density map of basal diameter for *Viburnum dilatatum*, and c) variance of basal diameter for *Viburnum dilatatum* within the macroplot. d) Density of *Viburnum sieboldii* individuals, e) density map of basal diameter for *Viburnum sieboldii*, and f) variance of basal diameter for *Viburnum sieboldii* within the macroplot.

Figure 18. Basal diameter of *Viburnum dilatatum* broken into three size class based on quartiles which can be used as an approximation for age. a) Late or older shrubs, b) mid- or middle-aged shrubs, and c) the early or young shrubs. d) Plot with all three size classes combined.

The most likely ecological explanation for the patterns exhibited by *V. dilatatum* at the local/plot level can be attributed to two dispersal factors: seedfall around shrub and dispersal by birds however, environmental factors cannot be completely ruled out. The first chapter illustrated how *V. dilatatum* is dispersed locally by birds, especially the American Robin (*Turdus migratorius*) and how the fruit persisted until winter. In fact, it
was not uncommon for fruit to never be consumed but rather abscise and fall near the parent plant (Fig. 19 - Anthony Cullen, personal observation).

Figure 19. Fruit from *Viburnum dilatatum* littering the ground. Individual fruit would abscise all the way up to a whole peduncle. Image taken February 17, 2016.

I believe this explains why the pair correlation (Fig. 11b) shows clustering from one to four meters due to a combination between seedfall and bird-dispersal and a regular (hyperdispersed) pattern from six to thirteen meters is likely due to localized bird-dispersal. Seed rain and seed shadow typically show a leptokurtic distribution of seed density (Portnoy and Willson 1993, Kollmann 2000, Nathan and Muller-Landau 2000, Levin et al. 2003), meaning more seeds fall closer to parent plant and fewer are found the further away you go. Indeed, studies that have looked at other bird-dispersed fleshy fruit have found that locations less than ten meters away from the parent plant have the highest
seed density, which can impact recruitment (McDonnell and Stiles 1983, Hoppes 1988).

In *V. dilatatum*’s native range in Japan, Takahashi studied seed rain of nine fleshy fruited plants, including *V. dilatatum* and found that plants with larger fruit yield and high fruit removal from birds had a high intensity of seed rain below the plant (Takahashi and Kamitani 2004) and when consumed by birds the seeds were more likely to be defecated by birds near other conspecific plants (Takahashi and Kamitani 2003). It is important to note that both Takahashi studies included *Turdus* spp, the Dusky Thrush (*Turdus naumanni*) and Pale Thrush (*Turdus pallidus*), as they were important frugivore dispersers. Wheelwright (1991) observed that it was common for birds to drop seeds below fruiting trees and often returned to trees to regurgitate or defecate from previous visits. His study included the Mountain Robin (*Turdus plebejus*), and found that they were important dispersers with an average visitation time in fruiting trees ranging from two to five minutes and they ate about one to three seeds per visit. Other studies highlight the importance of perch structures and how more seeds are found around perch sites as they act as recruitment foci (McDonnell and Stiles 1983, Debussche and Isenmann 1994, Kollmann and Pirl 1995, Holl 1998) or nucleation sites (Pausas et al. 2006). The implications of this means that birds are likely to use the same *V. dilatatum* individual it fed from or another *V. dilatatum* as perching sites therefore increasing the likelihood of *V. dilatatum* being tightly clustered around other conspecifics but also the reason why there is a hyperdispersed pattern at six to thirteen meters because birds like the American Robin (*Turdus migratorius*) are perching in adjacent *V. dilatatum* and spreading the seed through defecation. Indeed, McDonnell and Stiles (1983) suggested that the steep decay curve of seed dispersal had peaks after the initial decay that represented recruitment foci
(shrubs) where seed dispersal was high. A study from Jordano and Schupp (2000) found that birds only tended to fly approximately fifteen meters away from the plant they were feeding on. Their study also included *Turdus spp*, which are known seed dispersers, Mistle Thrush (*Turdus viscivorus*) and Common Blackbird (*Turdus merula*) and found they had high visitation and feeding rates which translated to a high probability of seed dispersal. Generally, they had short visitations and handling times (20 second and ~1 minute) but removed anywhere from six to nine fruits, most of which were carried away to another perch close by. Both of these studies would help explain the highly dispersed pattern I see in this dataset in conjunction with what the potential dispersal is like for *V. dilatatum* in the previous chapter.

I believe these explanations of the ecological dispersal patterns given above could explain why the mark correlation analysis for *V. dilatatum* showed that the basal diameter of individuals is uncorrelated at any distance (Fig. 13a) and the mark variogram suggests that individuals are generally similar in size to their nearest neighbor (Fig. 13b). If we accept the idea that seedlings will spread to nearby parent plant and adjacent conspecifics by seedfall and bird dispersal and that this in turn will enhance recruitment of *V. dilatatum*, then logically it would also follow that mature shrubs, saplings, and seedlings would associate closely, hence clustering. It is also a likely explanation for why there is no strong association between basal diameter as various age classes are associated together. This also would explain why the mark variogram results imply that the nearest neighbors’ basal diameters are uncorrelated at any distance because all different sized plants are mixed together. You can see this clearly when breaking the plot into size class by basal diameter in Figure 18 as all different size classes are found next to one another.
The inhomogeneous pair correlation for *V. sieboldii* individuals (Fig. 12b), again, generally supports what the density map shows (Fig. 17d) that *V. sieboldii* individuals are only clustered at distances from approximately one to three meters. This trend seems supported overall for the macroplot but the density map shows clustering of individuals near in the southern corner of the plot. Between three and eight meters the data falls within the simulation envelope indicating that at this distance they are randomly spaced. The data emerges below the border of the simulation envelope between eight and twelve meters suggests *V. sieboldii* spacing is dispersed (Fig. 12b). Again, the mark correlation analysis lends insight into how basal diameter affects the relationships among the individual *V. sieboldii* trees. For *V. sieboldii*, basal diameter of individuals is significantly larger than would be expected at distances from two meters and beyond, meaning stimulation (Fig. 15a), although they remain larger than average, the size does decrease with distances beyond eight meters. Another interpretation is that large trees associate with large trees and small trees associate with small trees. Otherwise individuals’ sizes less than two meters from the nearest neighbor are not correlated as the data falls within the simulation envelopes. The mark variogram also suggests that individuals are generally similar in size to their nearest neighbor, especially at distances approximately from zero to five meters supporting the idea that similar size classes are associating with each other (Fig. 15b). When breaking basal diameter into size class by quartile, this trend becomes apparent. Figure 20 shows late, mid-, or early classes based on their size, again, the assumption being that the larger the basal diameter the older the individual. The plot with only late or older shrubs (Fig. 20a) all associate or cluster in the same general area, the southern end of the plot. The mid- or middle-aged shrubs (Fig. 20b) tend to be found
north and east of the larger size class. The early or young shrubs (Fig. 20c) almost exclusively form a halo around the larger size class. This trend is not as obvious when all points are overlaid (Fig. 20d).

Figure 20. Basal diameter of *Viburnum sieboldii* broken into three size class based on quartiles which can be used as an approximation for age. a) Late or older trees, b) mid- or middle-aged trees, and c) the early or young trees. d) Plot with all three size classes combined.

The most likely ecological explanation for the patterns exhibited by *V. sieboldii* at the local/plot level can be attributed primarily to two dispersal factors: clonal/vegetative growth by individuals and dispersal by birds however, environmental factors cannot be completely ruled out. The first chapter illustrated how *V. sieboldii* fruit is readily eaten and dispersed by birds migrating in the fall, especially the Gray Catbird (*Dumetella carolinensis*). While this increased the probability of long-distance seed dispersal it also meant that birds were likely to spread them locally as well. However, it was not noted that *V. sieboldii* could also spread clonally by adventitious buds along the roots (root sucker) or shoots (Fig. 21 - Anthony Cullen, personal observation).
Figure 21. *Viburnum sieboldii* can easily spread asexually. a) A clonal stand of young *Viburnum sieboldii* next to one b) cut down and treated but yet it persisted and was able to produce new buds at the base. c) A branch from a sapling *Viburnum sieboldii* touches the ground where it sets adventitious roots. d) Close up of same branch dug out with arrow point to root. e) A *Viburnum sieboldii* was cut and laid down on the ground and was still able to root.

I believe this explains why the pair correlation (Fig. 12b) shows clustering from one to three meters largely due to a clonal growth and perhaps to a lesser extent bird-dispersal and the regular (hyperdispersed) pattern from eight to twelve meters is largely due to bird-dispersal. The mark correlation also supports clonal for *V. sieboldii* because large trees associate with large trees and small trees associate with small trees (Fig. 15a). This implies mutual stimulation of individuals or that they benefit from being close together (Illian et al. 2008, Szmyt 2014, Wiegand and Moloney 2014). In this case, I believe that similar size classes are associating because they established clonally, therefore if one plant establishes and then spreads clonal, that the clonal plants surrounding the first plant are likely to be the same size. Indeed, this is what we see in figure 20, that when broken into size classes, that within each class the plants cluster close to one another. Künstler (1993) also observed clonal behavior in *V. sieboldii*. In his
observation, he stated that next to a large *V. sieboldii* were many seedlings and smaller saplings, some that were vegetative. Over time, if one tree is the nucleation site for other trees, then the associations of size classes captured in the macroplot becomes clearer because you will find similar sized trees clustered together if they are mainly spreading vegetatively. The mark variogram maintains this idea as it shows that individuals are generally similar in size to their nearest neighbor, especially at distances approximately from zero to five meters (Fig. 15b).

However, it is still reasonable to assume that some of the dispersal is mediated by birds consuming *V. sieboldii* fruit. The pair correlation does show hyperdispersal from eight to twelve meters which is similar to the pattern exhibited by *V. dilatatum*. Gray Catbirds were shown to be the primary consumers of *V. sieboldii* fruit and therefore would mainly be responsible for that dispersal. Frugivorous birds have been shown to have short seed retention time (Herrera 1984, Karasov and Levey 1990) and Gray Catbirds are known seed dispersers (Stiles 1980) that can efficiently digest fruit (Johnson et al. 1985). So, while there might be a chance for long-distance seed dispersal it is unlikely in the case of Gray Catbirds as they migrate at night and stop over during the day to forage (Smith et al. 2011). If their gut retention times are short then most likely they are defecating the seeds locally which would explain the hyperdispersed pattern we see from eight to twelve meters. Especially, if their flight distance from the parent shrub is limited and they are using perch structures nearby.

**Patterns of dispersal and ramifications for invasion**

The patterns of distribution at the local/plot level in conjunction with the data from the first study give a more complete picture of the process of local dispersal. My
interpretation of the patterns for both species were based off of personal observation, a
literature review, and what the point pattern analyses seems to suggest. I think that *V. dilatatum* spreads by fruit at close distances, through seedfall around shrubs and at
greater distances is dispersed by birds. While *V. sieboldii* is more likely to spread clonally
at short distances and dispersed by birds at greater distances. These analyses imply
different strategies but it is unclear which strategy may lead to greater invasion potential.
From personal observations, both species can dominate a site over time. Perhaps a more
useful metric would be the time it takes to spread over a given area as an indication of the
species invasion potential. However, this study only provides an explanation of the
dispersion pattern.

Regionally we know that *V. sieboldii* is found in more counties and states than *V. dilatatum* but that certainly *V. dilatatum* is more populous in NJ. However, it remains
unclear how both species have spread and why they are so widely distributed. Is it due to
a single individual that was planted which has since dispersed and naturalized over long
distances to colonize multiple states or multiple individual plantings in multiple states
that have led to more localized dispersal? Viburnum are an economically important genus
in the ornamental business and are in high demand as ornamental plantings (Klingeman et al. 2014). It stands to reason that there are multiple plantings regionally, both in local
botanical gardens and arboretums as well as used in residential landscaping because of
viburnum’s ornamental charms. The literature review clearly captures instances were
both species were used in ornamental plantings as well as found in collections at gardens
and arboreta. Understanding the regional patterns and attributing the means of spread is
certainly a difficult problem to untangle. What can be gleaned from the literature is that
in at least three cases with *V. dilatatum* in Connecticut, Illinois, and Washington, D.C. and at least five cases with *V. sieboldii* in New Jersey, New York, Connecticut, Massachusetts, and Washington, D.C. that these species likely escaped from plantings or gardens. However, to truly understand their dispersal and how or if these populations are linked across the state or the region, a landscape genetics approach to the problem would be more useful. Recent studies in invasion ecology have used landscape and population genetics to understand how small populations of non-natives make the jump to larger naturalized populations (Whitney and Gabler 2008, Le Roux and Wieczorek 2009, Rollins et al. 2013). The third chapter will more fully explore the genetic component of dispersal on a local and regional level. Employing this molecular approach will give this dispersal study a firmer grasp on the structure of the population and landscape level genetic diversity. These insights can lead to better understanding of dispersal mechanisms and gene flow between populations.

**Conclusion**

This study suggests that the two species have different strategies when spreading locally at distances under four meters. *Viburnum sieboldii* most likely relies on spreading clonally at distances under three meters but is likely bird-dispersed at distances from eight to twelve meters. While *Viburnum dilatatum* likely spreads by fruit both at close distances under four meters by seedfall and by localized seed dispersal at distances between six and thirteen meters. On the state and regional level, the patterns of distribution are less clear but will be explored more fully in the next chapter looking specifically at the relatedness between populations.

**Acknowledgments**
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Appendix

Figure A1. These are distribution maps based off of literature and observations provided by the Early Detection and Distribution Mapping System (EDDMapS) from the University of Georgia, Center for Invasive Species and Ecosystem Health for a) Viburnum dilatatum and b) Viburnum sieboldii.
Chapter 3: The great garden escape: the role of microevolution in the invasion of two ornamental viburnums.

Abstract

It is important to understand the microevolutionary mechanisms that are involved in a successful non-native species invasion. Typically, a newly colonized population of a non-native species has low genetic diversity due to the founder effect. One way a population is able to recover from the founder effect is through admixture of conspecifics from different populations. Propagule dispersal allows gene flow to occur among populations that would otherwise be geographically isolated from one another. *Viburnum dilatatum* and *Viburnum sieboldii*, two non-native ornamental species that escaped cultivation, have rapidly expanded their range within the last 30 years in the New York metropolitan area, central New Jersey, and the greater Philadelphia area. While both are successful invaders little is known about the genetic structure among naturalized populations. The last two chapters demonstrated differences in dispersal between the two species: *V. sieboldii* has a probability for long-distance seed dispersal by fall migratory birds but mainly spreads clonally at a population level, whereas *V. dilatatum* is dispersed locally by resident birds in the winter. The goal of this chapter is to understand if the population genetic structure supports these conclusions. I hypothesize that *V. sieboldii* will have relatively higher gene flow among populations because of long-distance seed dispersal but *V. dilatatum* will have relatively higher gene flow within a population because *V. dilatatum* is spread locally, whereas *V. sieboldii* spread clonally within a population. To test this hypothesis, SNP’s (single nucleotide polymorphism) from a subsample of 16 individuals per species, representative of the naturalized populations in
New York City, central New Jersey, and Philadelphia were used to compare genetic diversity within and among populations. The $F_{st}$ value for *V. sieboldii* suggests that individuals between populations are freely experiencing gene flow. In contrast, the $F_{st}$ value for *V. dilatatum* suggests significant population structure. Tajima’s D suggests that *V. dilatatum* has overcome the founder effect and the population is expanding while *V. sieboldii* is still in a genetic bottleneck despite similar invasion timelines.

**Introduction**

Recently, invasion ecology has looked to the molecular techniques of landscape genetics to understand how small populations of non-natives transition to larger naturalized populations (Whitney and Gabler 2008, Le Roux and Wieczorek 2009, Rollins et al. 2013). Typically, a newly colonized population of a non-native species has low genetic diversity due to the founder effect (Nei et al. 1975, Sakai et al. 2001, Allendorf and Lundquist 2003, Dlugosch and Parker 2008). One way a population is able to recover from the founder effect is through admixture of conspecifics from different populations (Mooney and Cleland 2001, Lee 2002, Ellstrand and Schierenbeck 2006, Prentis et al. 2008, Culley and Hardiman 2009, Schierenbeck and Ellstrand 2009, Harrison and Larson 2014). Gaining insight into rapid microevolutionary changes in invasive species will allow us to understand the factors involved in colonization and spread (Sakai et al. 2001). These insights can lead to better management and restoration practices if the evolutionary mechanisms for successful spread are explicitly known (Sakai et al. 2001, Schierenbeck and Ellstrand 2009). However, empirical studies demonstrating this theory are generally lacking.
In the first chapter it was posited that *Viburnum sieboldii* may have the opportunity for long-distance seed dispersal because their fruit is consumed mainly by migratory birds, while *Viburnum dilatatum* seed dispersal may be limited because primarily resident birds are dispersing the seeds. In the second chapter, the point pattern analysis was interpreted to suggest that on a local scale that *V. sieboldii* most likely relies on spreading clonally at distances under 3 meters and bird-enhanced seed dispersal at distances greater than 8 meters. While *V. dilatatum* likely spreads by seed, both at distances under 4 meters due to seedfall and at distances greater than 6 meters by localized seed dispersal by birds. Based on the results from my first two chapters, I would predict more genetic variation within populations for *V. dilatatum* as they are spreading primarily by seed and therefore are likely to be freely experiencing gene flow among individuals within a population. However, I would expect that there is less admixture from individuals among populations of *V. dilatatum* because they are dispersal limited, therefore each population would be distinct from one another. For *V. sieboldii*, within populations I would expect less genetic variation because of clonal growth at a local scale but a higher probability that there could be admixture among populations of *V. sieboldii* because of the potential for long-distance seed dispersal.

The goal of this chapter is to understand if the population genetic structure supports these conclusions. I hypothesize that *V. sieboldii* will have relatively higher gene flow among populations because of long-distance seed dispersal but *V. dilatatum* will have relatively higher gene flow within a population because *V. dilatatum* is spread locally, whereas *V. sieboldii* spreads clonally within a population. To test this hypothesis, SNP’s (single nucleotide polymorphism) from a subsample of 16 individuals per species,
representative of the naturalized populations in New York City, central New Jersey, and Philadelphia were used to compare genetic diversity within and among populations. Genetic structure is discussed with reference to the dispersal strategies of the two species of *Viburnum*.

**Methods**

*Study sites and sampling protocols*

Leaf samples were collected from May through August of 2016 across study sites in New York, New Jersey, and Pennsylvania. Samples were collected in multiple town, county, state, and federal parks where one or both viburnum species have naturalized populations. Samples were also collected in nearby botanical gardens or arboretum collections to see if naturalized populations could be traced back to these cultivated specimens (Table 1a, b and Fig.1). In total, 300 individuals were sampled across the study area, 140 *Viburnum dilatatum* and 160 *Viburnum sieboldii*. Approximately four to six leaves were collected per individual and were immediately placed in coin envelopes with indicating silica gel with a ratio of 10:1 silica gel to leaf mass as a preservation method for DNA extraction (Chase and Hills 1991). On each envelope the plant location, taxonomic name, sample number, collector name, and date were recorded for all samples. Envelopes were placed in an air and water-tight, resealable container with indicating silica gel and then placed in a cabinet to exclude light to preserve leaf material until DNA extraction.

**Table 1. Location and sample size of cultivated and naturalized a) *Viburnum dilatatum* and b) *Viburnum sieboldii*.**
### Viburnum dilatatum

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<th>Total</th>
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*Not present, known, or detected during sampling

### Viburnum sieboldii

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*Not present, known, or detected during sampling
Figure 1. Regional map of sample site locations. a) Four sites located in New York City, NY across Bronx and Queens County; three parks with naturalized populations and one botanical garden for cultivated specimens. b) Nine sites in central New Jersey spread out across Morris, Essex, and Union County; six parks with naturalized populations and three arboreta/garden for cultivated specimens. c) Five sites in the greater Philadelphia and Trenton area. One in Mercer County in NJ and the others in Delaware, Montgomery, and Philadelphia County; one park with a naturalized population and four arboreta/garden for cultivated specimens.

**DNA extraction and subsample selection for RAD sequencing**

A QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc, Germantown, Maryland, USA) was used to extract DNA from preserved leaf samples. Due to the nature of preservation, a modified protocol was used that yields more DNA per extraction for herbarium specimens; detailed methods can be found in Drábková extraction methods 3.1 (2014). Once extracted, DNA samples were stored in a -20ºC freezer (Frigidaire, Augusta, Georgia, USA). DNA concentrations of all 300 individuals were quantified with a Qubit 3 Fluorometer and Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sample requirements for nextRAD
sequencing (Restriction site Associated DNA) with SNPsaurus (SNPsaurus LLC, Eugene, Oregon, USA) required purified genomic DNA concentrations of 5 ng/µl. A subsample of 32 individuals, 16 per species, representative of the naturalized populations and arboreta collections in New York, New Jersey, and Pennsylvania were selected (Table 2). The specific extracts to be analyzed for SNPs were chosen to represent various sizes of shrubs within the population, therefore basal diameter of the shrub was used as a proxy. SNPsaurus required quality control standards of all individuals in subsamples and were assessed quantitatively with the Qubit 3 Fluorometer to ensure that samples were at or above the 5 ng/µl DNA concentrations and assessed qualitatively by gel electrophoresis to ensure the DNA was not degraded. 50 µl per sample were pipetted into PCR strip tubes and sealed with flat strip caps. Tubes were placed into a 96 well plate and wrapped in parafilm to prevent movement during shipping as well as leakage of subsample. Samples were placed on dry ice in an insulated container for shipment to SNPsaurus. SNPsaurus prepared and sequenced a nextRAD library with 30,000 loci sequenced to 5X read depth with 150 bp reads. This type of genotyping-by-sequencing (GBS) with nextRAD is used for single nucleotide polymorphism (SNP) discovery. The next section goes into details about the protocols SNPsaurus implemented to acquire the SNP data.

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SNPsaurus protocol

Genomic DNA was converted into nextRAD genotyping-by-sequencing libraries (SNPsaurus, LLC) as in Russello et al. (2015). Genomic DNA was first fragmented with Nextera reagent (Illumina, Inc, San Diego, California, USA), which also ligates short adapter sequences to the ends of the fragments. The Nextera reaction was scaled for fragmenting 20 ng of genomic DNA, although 40 ng of genomic DNA was used for input to compensate for the amount of degraded DNA in the samples and to increase fragment sizes. Fragmented DNA was then amplified for 26 cycles at 73°C, with one of the primers matching the adapter and extending 9 nucleotides into the genomic DNA with the selective sequence GTGTAGAGC. Thus, only fragments starting with a sequence that can be hybridized by the selective sequence of the primer will be efficiently amplified. The nextRAD libraries were sequenced on a HiSeq 4000 with one lane of 150 bp reads (University of Oregon).

The genotyping analysis used custom scripts (SNPsaurus, LLC) that trimmed the reads using bbduk ((BBMap tools, http://sourceforge.net/projects/bbmap/):bviburnum bbmap/bbdsk.sh in=$file out=$outfile ktrim=r k=17 hdist=1 mink=8 ref=bbmap/resources/nextera.fa.gz minlen=100 ow=t qtrim=r trimq=10; date last accessed: 10-20-17). Next, a de novo reference was created by collecting 10 million reads

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*Sample was too contaminated with other DNA to be used in study
in total, evenly from the samples, and excluding reads that had counts fewer than 7 or more than 700. The remaining loci were then aligned to each other to identify allelic loci and collapse allelic haplotypes to a single representative. All reads were mapped to the reference with an alignment identity threshold of 85% using bbmap (Bushnell 2014). Genotype calling was done using Samtools and bcftools (samtools mpileup -gu -Q 12 -t DP,DPR -f ref.fasta -b samples.txt | bcftools call -cv - > genotypes.vcf) (Li et al. 2009, Li 2011). The vcf file (Danecek et al. 2011) was filtered to remove alleles with a population frequency of less than 5%. Loci were removed that were heterozygous in all samples or had more than 2 alleles in a sample (suggesting collapsed paralogs). The absence of artifacts was checked by counting SNPs at each read nucleotide position and determining that SNP number did not increase with reduced base quality at the end of the read.

**Data processing and analysis**

SNPsaurus returned a vcf file containing the genotype table generating 3813 SNP sites for analysis and a PHYLIP file (PHYlogeny Inference Package) containing a 61650 long nucleotide sequence for each of the 32 samples. Genotypic data were used for all analysis to compare the genetic diversity of populations for both species of viburnum. A maximum likelihood phylogenetic analysis was performed on a dataset including SNPs from both species on the IQ-TREE web-server (http://iqtree.cibiv.univie.ac.at) (Nguyen et al. 2015, Trifinopoulos et al. 2016) using an standard bootstrap analysis (Felsenstein 1985). The best model for statistical analysis with was determined using ModelFinder (Kalyaanamoorthy et al. 2017) in IQTREE, and was found to be TVM+F+I+G4 for *Viburnum dilatatum* and TVM+F+G4 for *Viburnum sieboldii*. The number of parsimony-informative characters was estimated. The standard bootstrap analysis was run with 100
bootstrap alignments. Results were downloaded from IQ-TREE web server and consensus tree files were visualized for both species in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and figures were edited in Inkscape (Harrington, B. et al 2004-2005, Inkscape, https://inkscape.org/en/). Haplotype networks were reconstructed in PopART 1.7 (POPulation Analysis with Reticulate Trees) (Bandelt et al. 1999, Leigh and Bryant 2015) to visually assess and compare genetic diversity of individuals among populations as a complement to the phylogenetic analyses. For each species, several population genetic statistics, the Analysis of Molecular Variance (AMOVA) and Tajima’s D, were calculated for all the naturalized populations using Arlequin 3.5.2.2 (Excoffier and Lischer 2010). AMOVA (Weir and Cockerham 1984, Excoffier et al. 1992, Weir 1996) detects population differences using $F_{ST}$ values to compare genetic diversity among and within populations. $F_{ST}$ values range from 0 to 1, with $F_{ST}$ values closer to 0 indicating populations are freely experiencing gene flow, and $F_{ST}$ values closer to 1 indicating populations are experiencing low gene flow. Population assignments for each species assumed that populations were geographically isolated from one another. For *V. dilatatum*, the following populations were assigned: Cunningham Park (NY), Washington Crossing (southern NJ), and the Morristown Parks (Lewis Morris County Park and Morristown National Historical Park) (northern NJ). For *V. sieboldii* the following populations were assigned: Morris Woods (PA), Cunningham Park (NY), Washington Crossing (southern NJ), and the Morristown Parks (Fosterfields Living Historical Farm, Lewis Morris County Park and Morristown National Historical Park) (northern NJ). Morristown parks were considered one population due to the close proximity of parks. The AMOVA ran 1000 permutations and the population pairwise $F_{ST}$
values ran 1000 permutations for the Mantel test and 100 permutations for significance with an alpha of 0.05. Tajima’s D (Tajima 1989, Tajima 1996) considers the number of nucleotide sites that differ between individuals, which is useful for understanding the amount of variation in a population. A negative Tajima’s D suggests recent population expansion after a bottleneck event and a positive Tajima’s D suggests recent population contraction or a population experiencing a genetic bottleneck (Rand 1996). A bottleneck occurs when a population size is drastically reduced, this loss negatively affects the overall genetic diversity of the remaining population (Futuyma 2009). Parameters for Tajima’s D are $S$, the number of segregating sites (i.e. polymorphisms or variable sites) for population as a whole, $\pi$, the average pairwise difference between individuals, and $\theta$, the expectation of $\pi$. Tajima’s D is calculated by taking the observed differences minus the expected ($\pi - \theta$) to understand the variation in the population. Tajima’s D was calculated within populations for each species as well as cumulatively across all populations and was run for 1000 permutations. Structure 2.3.4 (Pritchard et al. 2000, Falush et al. 2003, 2007, Hubisz et al. 2009) plots were used to visually represent the admixture between populations as well as determine the true number of populations per species. The analysis assumed the number of potential populations ranging from 1 to 10, each population ($K$) was run with 10 iterations and a Markov chain Monte Carlo was run 10000 times per population with a 1000 burn-in per simulation. Results were run through Structure Harvester (Earl and vonHoldt 2012) and CLUMPACK (CLUster Markov Packager Across $K$) (Kopelman et al. 2015) to get a true estimate of population size ($K$) by the Evanno method of Delta$K$ (Evanno et al. 2005). However, the Evanno method is only suitable for analysis when the assumed number of populations are two or more. If
the assumed population is one then the Evanno method could not be used for population estimation, instead we use the measure \( L(K) \) (aka \( \text{Ln P(D)} \)) which is the posterior probability for a given \( K \) (population). The maximal mean \( L(K) \) will be accepted as the true population number.

**Results**

*IQ-TREE maximum likelihood, phylogeny, and haplotype networks*

Using IQ-TREE, we analyzed the 3813 SNP sequence for 16 *Viburnum dilatatum*, of which 3020 were constant or invariant sites (79.20% of all sites), 422 were parsimony-informative, 371 were singleton sites, and 1861 were distinct site patterns. The best fit model for *V. dilatatum* was TVM+F+I+G4 or Tranversion model for base substitution rates where AG=CT+empirical base frequencies for unequal base frequencies+allowing for a proportion of invariable sites+gamma distribution with four categories for the rate of heterogeneity across sites. IQ-TREE analyzed the 3813 SNP sequence for 15 *Viburnum sieboldii*, of which 2867 were constant or invariant sites (75.19% of all sites), 471 were parsimony-informative, 475 were singleton sites, and 242 were distinct site patterns. The best fit model for *V. sieboldii* was TVM+F+G4 or Tranversion model for base substitution rates where AG=CT+empirical base frequencies for unequal base frequencies+gamma distribution with four categories for the rate of heterogeneity across sites.

The reconstructed topology of *V. dilatatum* recovers one monophyletic clade that has many polytomies (Fig 2a), with three subclades that are not well supported and one outgroup. The outgroup is an individual from the New York Botanical Garden that is a cross between *V. dilatatum* and *V. lobbophyllum* that is a cultivar called ‘Oneida’. The first
subclade has an individual from Morristown National Historical Park that is a sister
group to individuals from Cunningham Park and Morris Arboretum with a low supported
bootstrap value of 36%. That individual from Cunningham Park is a sister group to
another individual from Cunningham Park and Morris Arboretum with a low supported
bootstrap value of 29%. The two individuals from Cunningham Park and Morris
Arboretum are a sister group with support of 66%. The second subclade are all
individuals from Washington Crossing State Park that are a sister group with low
supported bootstrap ranging from 13% to 44%. The third subclade has a sister group with
two individuals from Cunningham with low supported bootstrap of 43%. Those
individuals are a sister group to individuals from Morristown National Historical Park,
Frelinghuysen Arboretum, and Cunningham Park with a low supported bootstrap value of
12%. That individual from Morristown National Historical Park is a sister group to an
individual from Frelinghuysen Arboretum and two individuals from Cunningham Park
with a low supported bootstrap value of 40%. The individual from Frelinghuysen
Arboretum is a sister group to the two individuals from Cunningham Park with a
supported bootstrap value of 68% and the two individuals from Cunningham Park are a
sister group to each other with a supported bootstrap value of 60%. The haplotype
network for *V. dilatatum* illustrates that there are very few site differences between all
individuals except for the *V. dilatatum* ‘Oneida’ from the New York Botanical Garden
with thirteen site differences. All other individuals range from one to six site differences
with all individuals from Washington Crossing State Park have relatively the least
number of site differences.
Figure 2. Phylogeny and haplotype network for *Viburnum dilatatum*. a) Topology shows one monophyletic clade, with three subclades that are not well supported. b) Haplotype network shows the greatest similarities among individuals at Washington Crossing State Park while all other individuals from other populations range between two and six site differences.

The reconstructed topology for *V. sieboldii* recovers one monophyletic clade that has many polytomies (Fig 3a), with two subclades that are not well supported and two
unresolved individuals from Morristown National Historical Park and the New York Botanical Garden. In the first subclade an individual from Morris Woods is a sister group to individuals from Morristown National Historical Park, Fosterfields Living Historical Farm, and another individual from Morris Woods with a low supported bootstrap value of 27%. That individual from Morristown National Historical Park is a sister group to individuals from Fosterfields Living Historical Farm and Morris Woods with a low supported bootstrap value of 15%. Those two individuals are sister to each other with a low supported bootstrap value of 36%. Within the second subclade is an individual from Morris Woods that is a sister group to two individuals from Washington Crossing State Park and one from Cunningham Park with a low supported bootstrap value of 13%. One of the individuals from Washington Crossing State Park is a sister group to an individual from Cunningham Park and another individual from Washington Crossing State Park with a low supported bootstrap value of 35%. The individual from Cunningham Park and Washington Crossing State Park are a sister group with a low supported bootstrap value of 30%. This first group of individuals are sister to another group of individuals from Washington Crossing State Park, Lewis Morris County Park, Morristown National Historical Park, and Cunningham Park with a low supported bootstrap value of 11%. That individual from Washington Crossing State Park is a sister group to individuals from Lewis Morris County Park, Morristown National Historical Park, and Cunningham Park with a supported bootstrap value of 62%. That individual from Lewis Morris County Park is a sister group to an individual from Morristown National Historical Park and two individuals from Cunningham Park with a low supported bootstrap value of 39%. The individual from Morristown National Historical Park is a sister group to the two
individuals from Cunningham Park with a low supported bootstrap value of 25% and the two individuals from Cunningham Park are a sister group with each other with a supported bootstrap value of 50%. The haplotype network for *V. sieboldii* illustrates that there are very few site differences between all individuals, typically between two and six.

Figure 3. Phylogeny and haplotype network for *Viburnum sieboldii*. a) Topology shows one monophyletic clade, with two subclades that are not well supported. b) The haplotype network shows that differences between all individuals regardless of assumed populations range between
two to six site differences.

**AMOVA and Tajima’s D**

The AMOVA for *V. dilatatum* had 1373 usable loci for the distance computation with 5% allowed level of missing data out of 3813 possible loci. The $F_{ST}$ value for *V. dilatatum* was 0.15826 with a significant p-value of $0.02835 \pm 0.00505$, which suggests significant genetic differentiation or population structure. Among populations the percent of variation is 15.83 and within populations the percent of variation is 84.17 (Table 3). The pairwise $F_{ST}$ values comparing pairs of populations found that the populations from Cunningham Park and Washington Crossing Park are freely crossing with the populations from Morristown Parks. However, Cunningham Park and Washington Crossing State Park populations are genetically distinct from one another (Table 4). The Tajima’s D analysis for all populations of *V. dilatatum* found 27 segregating sites with a Tajima’s D value of $-0.145571$ with a p-value of $0.538392$, which suggests a recent population expansion after a bottleneck event. The Tajima’s D values comparing the amount of variation in a population found that all populations had a negative Tajima’s D, which suggests the population is recovering from a bottleneck event (Table 5).

Table 3. AMOVA for naturalized populations of *Viburnum dilatatum* suggests panmixia.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>$F_{ST}$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>2</td>
<td>13.412</td>
<td>0.69661</td>
<td>15.83</td>
<td>0.15826</td>
<td>0.02835±0.00505</td>
</tr>
<tr>
<td>Within populations</td>
<td>10</td>
<td>37.050</td>
<td>3.70500</td>
<td>84.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>50.462</td>
<td>4.40161</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. AMOVA comparing pairs of naturalized populations for *Viburnum dilatatum*. Text highlighted in green is significant.
Table 5. Tajima’s D for naturalized populations of *Viburnum dilatatum* suggests a recent population expansion after a bottleneck event.

<table>
<thead>
<tr>
<th>Tajima's D-Viburnum dilatatum</th>
<th>Intra-population Level</th>
<th>Inter-population Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistics</td>
<td>Cunningham Park</td>
<td>Morristown Parks</td>
</tr>
<tr>
<td>Sample size</td>
<td>107</td>
<td>110</td>
</tr>
<tr>
<td>S</td>
<td>107</td>
<td>110</td>
</tr>
<tr>
<td>Pi</td>
<td>58.167</td>
<td>58.833</td>
</tr>
<tr>
<td>Tajima’s D</td>
<td>-0.204</td>
<td>-0.208</td>
</tr>
<tr>
<td>Tajima's D p-value</td>
<td>0.592</td>
<td>0.503</td>
</tr>
</tbody>
</table>

The AMOVA for *V. sieboldii* had 853 usable loci for the distance computation with 5% allowed level of missing data out of 3813 possible loci. The F<sub>ST</sub> value for *V. sieboldii* was 0.07264 with a p-value of 0.12219±0.001037, which suggest populations are freely crossing (panmictic). Among populations the percent of variation is 7.26 and within populations the percent of variation is 92.74 (Table 6). The pairwise F<sub>ST</sub> values comparing pairs of populations found that all population pairs are freely crossing (Table 7). The Tajima’s D analysis for all populations of *V. sieboldii* found 22 segregating sites with a Tajima’s D value of 0.598234 with a p-value of 0.569676, which suggests a recent population contraction or a population in a bottleneck. Interestingly, the Tajima’s D values comparing the amount of variation in a population found that each population was in equilibrium except for Morristown Parks that had a negative Tajima’s D, which suggests the population is recovering from a bottleneck event (Table 8).

Table 6. AMOVA for naturalized populations of *Viburnum sieboldii* suggests panmixia.
Table 7. AMOVA comparing pairs of naturalized populations for *Viburnum sieboldii*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>FST</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>3</td>
<td>14.157</td>
<td>0.29139</td>
<td>7.26</td>
<td>0.07264</td>
<td>0.12219±0.01037</td>
</tr>
<tr>
<td>Within populations</td>
<td>10</td>
<td>37.200</td>
<td>3.72000</td>
<td>92.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>51.357</td>
<td>4.01139</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Tajima’s D for naturalized populations of *Viburnum sieboldii* suggests a recent population contraction or a population in a bottleneck.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Cunningham Park</th>
<th>Morristown Parks</th>
<th>Washington Crossing State Park</th>
<th>Morris Woods</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunningham Park</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morristown Parks</td>
<td>0.02331</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washington Crossing State Park</td>
<td>-0.08197</td>
<td>0.04807</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris Woods</td>
<td>0.12000</td>
<td>0.14876</td>
<td>0.13158</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Structure

Using the Evanno method calculated in Structure Harvester, it was found that there are likely two populations for *V. dilatatum* (Fig. 4) as the DeltaK was highest at 1.784133, at a K of 2. The Evanno method is based on the rate of change in the log probability of K rather than just the log probability of K, Evanno determined DeltaK is a better predictor of populations (clusters) (Evanno et al. 2005). The Structure bar plots represents how these populations breakdown per individual at each sample site (Fig. 5a), by Q-values (Fig. 5b), and the accompanying table (Table 9) gives the proportion of Q-values which represent the cluster membership coefficient (i.e. probability that an
individual belongs to one or more populations through admixture). The four individuals from Morristown Parks are closely related to the four individuals from Cunningham Park, however, the proportion of Q-values differ with two individuals from Morristown Parks and Cunningham Park having more proportion of cluster 1 while the other two individuals from Morristown Parks and Cunningham Park have more proportion of cluster 2 (Fig. 5b and Table 9). All four individuals from Washington Crossing State Park have very similar proportions of Q-values.

\[
\text{Delta } K = \frac{\text{mean}(|L''(K)|)}{sd(L(K))}
\]

Figure 4. Evanno method to determine K (populations) by calculating the peak Delta K for *Viburnum dilatatum*. Two is the optimal number of populations for *Viburnum dilatatum* according to the Evanno method.
Figure 5. Structure bar plot for *Viburnum dilatatum*. a) Structure plot organized by location and b) A Structure plot organized by the Q-value.

Table 9. Q-values for *Viburnum dilatatum* that relate to the structure bar plot.
Structure found that there is likely only one population for *V. sieboldii*. The maximal mean $L(K)$ was -32597.91 with a standard deviation of 28.274073 (Fig. 6). Therefore, the resulting Structure bar plot shows that all Q-values are the same between individuals within and between sample locations or no admixture.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Morristown Parks</th>
<th>Cunningham Park</th>
<th>Washington Crossing State Park</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1 (Q-values)</td>
<td>0.399 0.505 0.711 0.206</td>
<td>0.885 0.463 0.341 0.919</td>
<td>0.090 0.078 0.080 0.217 0.085</td>
</tr>
<tr>
<td>Cluster 2 (Q-values)</td>
<td>0.601 0.495 0.289 0.794</td>
<td>0.115 0.537 0.659 0.081</td>
<td>0.910 0.922 0.920 0.783 0.915</td>
</tr>
</tbody>
</table>

Figure 6. The maximal mean $L(K)$ is -32597.91 for *Viburnum sieboldii*, which is suggest there is only one population.
Discussion

When comparing the phylogenies, we see that both species are monophyletic clades that are not well supported. The only distinguishable difference is that *Viburnum dilatatum* has three distinct subclades (Fig. 2) while *Viburnum sieboldii* has only two (Fig. 3). The phylogeny of *V. dilatatum* shows that the first subclade has individuals from the Morristown Parks (Lewis Morris County Park and Morristown National Historical Park) with close associations with the specimen from the Morris Arboretum. While the second subclade has all individuals from Washington State Crossing Park are a sister group to each other. The third subclade has all the individuals from Cunningham Park, one from Morristown National Historical Park, along with a specimen from Frelinghuysen Arboretum. This is interesting because the individuals from populations in the New York and central New Jersey have close associations while the southern New Jersey population seems isolated, except for the specimen from Morris Arboretum in Pennsylvania that has associations to individuals from the Morristown Parks. The haplotype network also makes this distinct that all individuals from Washington Crossing

![Figure 7. Structure bar plot for *Viburnum sieboldii* organized by location.](image)
State Park are similar. The phylogeny and the haplotype network both serve to show that the specimen from the New York Botanical Garden serves as an outgroup and an outlier, respectively. This is due to the fact that this specimen is a cultivar called ‘Oneida’ which is a cross between *V. dilatatum* and *V. lobophyllum* (Egolf 1956, Egolf 1966, Dirr 1990, 2007), both from the Old World *Odontotinus* clade (Donoghue et al. 2004, Winkworth and Donoghue 2004, 2005), and therefore is more distantly related to wild type *V. dilatatum* because it is a hybrid. In general, many cultivars would vary genetically from the wild type because they are selected and propagated for a desirable phenotype that is differ than that of the wild type species or they are hybrids with other species (Brandenburg and Schneider 1988, Brickell et al. 2016). The other two cultivated individuals from Frelinghuysen and Morris arboretums are likely more similar to the naturalized populations as they are not cultivars.

*V. sieboldii*, on the other hand, is a monophyletic clade with many polytomies. The haplotype network demonstrated that all individuals, regardless of location, are similar to one another. Cultivated and naturalized individuals were included when doing the phylogenies and haplotype networks for both species to get a sense if naturalized populations were more closely related to specimens found at nearby botanical gardens. It is possible that there could be multiple locations where introductions occurred due to specimens in the collections at nearby botanical gardens “escaping” and that naturalized populations near these cultivated specimens would be more closely related because of the nature of the seed shadow from localized dispersal (Portnoy and Willson 1993, Kollmann 2000, Nathan and Muller-Landau 2000, Levin et al. 2003). However, it does not appear to be the case that specimens from arboreta are closely related to nearby naturalized
populations as the phylogenies and haplotype networks do not fully support this conclusion in every case but rather bird-enhanced seed dispersal is a more likely explanation. It is also important to consider that despite the fact that there are numerous cultivated specimens spread out across New York, New Jersey, and Pennsylvania it does not mean that they are genetically unique individuals. Most cultivated species are vegetatively propagated to retain desirable traits and are therefore clones of the original species or cultivar (Dirr 2007).

When calculating $F_{ST}$ values, cultivated individuals were removed from the analysis as you cannot do population comparisons with only one individual. $F_{ST}$ values for naturalized populations for *V. dilatatum* indicated that there was significant population structure. While $F_{ST}$ values for naturalized populations for *V. sieboldii* indicated that populations are freely crossing (panmixia/panmictic populations). This outcome does support the original hypothesis that *V. sieboldii* would have relatively higher gene flow between different populations of conspecifics than *V. dilatatum* due to the differences in bird-enhanced dispersal. The pairwise comparison does illustrate that *V. dilatatum* has two distinct populations and *V. sieboldii* has one large population. For *V. dilatatum*, the population from Cunningham Park is significantly different from the population from Washington Crossing State Park but the comparison between all the other parks shows no significant differences between the populations. The Structure analysis does support that there are two populations of *V. dilatatum*. For *V. sieboldii*, there is no significant distinction between any of the populations with the pairwise comparison. The Structure analysis also supports the $F_{ST}$ values that there is one big population between all locations for *V. sieboldii*. Tajima’s D for each species offers some
additional insights into the changes in the population structure. The inter-population Tajima’s D for \textit{V. dilatatum} was -0.145, while it was not significant (Simonsen et al. 1995), a negative value does suggest a recent population expansion after a bottleneck event (Rand 1996). Plus, the intra-population Tajima’s D for each \textit{V. dilatatum} population were negative as well. While Tajima’s D for \textit{V. sieboldii} was 0.598, again while it was not significant (Simonsen et al. 1995), suggests recent population contraction or a population in a bottleneck (Rand 1996). However, the intra-population Tajima’s D for each \textit{V. sieboldii} showed that all populations were in equilibrium except for the Morristown Parks population which was a negative value, suggesting a recent population expansion. If we accept this interpretation based on Tajima’s D, it is likely that \textit{V. dilatatum} populations in New York and central New Jersey have already gone through a bottleneck event associated with the founder effect for a newly colonized species while the population from southern New Jersey and all populations of \textit{V. sieboldii} generally have not.

So, what then is going on? Why is \textit{V. dilatatum} split into two populations, one from central New Jersey and New York, and a separate population in southern New Jersey while \textit{V. sieboldii} is only one large population? What is the barrier to gene flow between Cunningham Park and Washington Crossing State Park for \textit{V. dilatatum}? Why does \textit{V. dilatatum} appear to have gone through a bottleneck event when \textit{V. sieboldii} has not? Especially given the fact that they both have very similar timelines for invasion. When considering distance alone, Cunningham Park is approximately 58 km away from the parks in Morristown and the Morristown Parks are only 65 km away from Washington Crossing State Park. However, Cunningham Park and Washington Crossing
Park are 104 km away. Why then, would parks 65 km or less be more genetically similar than a park another 104 km away? There could be several factors at play that would explain these differences that fit into two categories: population dynamics and dispersal. Population dynamics, such as how long the population has been established within a park and time to seed bearing age (aka sexual maturity) could help explain why the Tajima’s D results that show that *V. dilatatum* populations are recovering and the *V. sieboldii* populations are not. For dispersal, understanding modes of dispersal, dispersal vectors, and distance associated with the dispersal events could help explain why there are barriers to gene flow for *V. dilatatum* but not *V. sieboldii* demonstrated in the AMOVA results. Unfortunately, it is hard to gauge how long each population has been established in a particular location as detailed records of a species introduction are sometimes hard to come by. Even if this data was readily available we still would not have a clear understand of how many generations it would take these species of viburnum to overcome the founder effect. Plus, this is the first study to explore the genetic structure of these two species; there is no data that exists to compare how these populations have changed with each generation. However, a proxy can be used which is time it takes for each shrub to reach sexual maturity. We can also address the factors dealing with dispersal that have already been explored in the first two chapters.

While unfortunately no literature was found that detailed the age to sexual maturity for *V. dilatatum* and *V. sieboldii*. Bonner (2008) discusses in the ‘Woody Plant Seed Manual’ that native viburnums range in the time it takes to reach seed-bearing age from two to ten years and that in general, most species bloom every year after reaching that age. Bonner provides a table that lists the species, their growth habit (shrub or tree),
height at maturity, seed-bearing age, and years between seed crops. It seems that
difference between growth form (shrub or tree) and the height at maturity play a role in
Table 9. Table from Bonner’s ‘Woody Plant Seed Manual’ that describes the time it takes native viburnums to reach seed-bearing age.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth habit</th>
<th>Height at maturity (m)</th>
<th>Year first cultivated</th>
<th>Seed-bearing age (yrs)</th>
<th>Years between large seedcrops</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. acerifolium</td>
<td>Erect shrub</td>
<td>2</td>
<td>1736</td>
<td>2-3</td>
<td>1</td>
</tr>
<tr>
<td>V. dentatum</td>
<td>Erect shrub</td>
<td>5</td>
<td>1736</td>
<td>3-4</td>
<td></td>
</tr>
<tr>
<td>V. lantana</td>
<td>Shrub or tree</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V. lantanoides</td>
<td>Erect or trailing shrub</td>
<td>3</td>
<td>1820</td>
<td>—</td>
<td>3 or 4</td>
</tr>
<tr>
<td>V. lentago</td>
<td>Shrub of tree</td>
<td>10</td>
<td>1761</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>V. nudum var. nudum</td>
<td>Shrub or tree</td>
<td>1.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V. nudum var. cassioides</td>
<td>Erect shrub</td>
<td>3</td>
<td>1761</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>V. opulus</td>
<td>Erect shrub</td>
<td>4</td>
<td>—</td>
<td>3-5</td>
<td>—</td>
</tr>
<tr>
<td>V. pannorum</td>
<td>Shrub or tree</td>
<td>5</td>
<td>1727</td>
<td>8-10</td>
<td>1</td>
</tr>
<tr>
<td>V. rafinesquianum</td>
<td>Shrub</td>
<td>2</td>
<td>1830</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V. recognitum</td>
<td>Erect shrub</td>
<td>3</td>
<td>—</td>
<td>5-6</td>
<td>—</td>
</tr>
<tr>
<td>V. rufidulum</td>
<td>Shrub or tree</td>
<td>3.5</td>
<td>—</td>
<td>5</td>
<td>—</td>
</tr>
</tbody>
</table>

Source: Gill and Rogge (1974).

the time it takes to reach seed-bearing age, with tall shrubs and trees taking longer to bear seeds. If this is applicable to *V. dilatatum* and *V. sieboldii* than we would expect *V. dilatatum* to have shorter generation times. This is because *V. dilatatum* is shrub that reaches 3 meters in height when fully mature, while *V. sieboldii* is a subcanopy tree that can reach 6 meters in height when fully mature. Generation time would explain how one species would overcome the founder effect quicker than the other despite similar invasion timelines because the rates of evolution would be different between the two shrubs. Smith and Donoghue (2008), came to this conclusion when comparing the life history traits of angiosperms and found that plants with shorter generation times had higher rates of molecular change compared to that of plants with longer generation times. This seems the likeliest explanation as to why *V. dilatatum* has overcome the genetic bottleneck and why *V. sieboldii* has not.
Another possible explanation into why naturalized populations of *V. dilatatum* are recovering could be attributed to the difference in the number of cultivars between the two species: *V. dilatatum* has anywhere from nine (Klingeman et al. 2014) to sixteen (Dirr 2007) cultivars depending on the reference, whereas *V. sieboldii* only has two (Klingeman et al. 2014) to three (Dirr 2007). Each arboreta or botanical garden could have any permutation on the number of individual shrubs and cultivar types. Simply put, cultivated *V. dilatatum* would have a better chance at increasing genetic diversity in their offspring than *V. sieboldii* because it has anywhere from three to five times as many distinct cultivars to cross with. Plus, there would be multiple introductions associated with each crossing. Having diverse offspring and multiple introductions would help *V. dilatatum* when initially escaping cultivation and colonizing forest understories. Naturalized populations of *V. dilatatum* would also benefit from this admixture when expanding their populations (Verhoeven et al. 2011, Rius and Darling 2014). These explanations could certainly account for the Tajima’s D results. Indeed, there are many studies illustrating that multiple introductions and crossing with distinct haplotypes that might never occur in the native populations can increase genetic diversity (Genton et al. 2005, Lavergne and Molofsky 2007, Facon et al. 2008, Kolbe et al. 2008, Rosenthal et al. 2008, Pairon et al. 2010).

So how is gene flow happening between populations of conspecifics? Chapter one demonstrated that these species rely on bird-enhanced seed dispersal and there are differences in the species consuming the fruit and the timing of when the fruit is consumed; with *V. dilatatum* fruit dispersed locally by winter resident birds while *V. sieboldii* fruit dispersed locally and potential long distances by migratory birds. If we
accept that *V. sieboldii* is not dispersal limited than there is less of a barrier to gene flow between population. This would explain why *V. sieboldii* is panmictic. If we also accept that *V. dilatatum* isn’t likely dispersed over long distances it would explain the barrier to gene flow between the population from southern New Jersey and the populations in central New Jersey and New York. However, if *V. dilatatum* is dispersal limited, why than does gene flow seem to occur between populations in central New Jersey and New York? It is assumed that during the winter American Robins are eating the fruit and dispersing the fruit locally because American Robins are known to wander during the winter and forage for fruits (Vanderhoff et al. 2016). Although it may be incorrectly assumed that these birds were residents as migrants move up the into mid-Atlantic as early as February and March (Tyler 1949). Unfortunately, the dynamics of how populations of Robins move, whether migratory or local wanderings, is poorly understood (Vanderhoff et al. 2016). Although, if migrants, it might explain why gene flow occurs between New York and New Jersey populations.

**Conclusion**

Despite similar invasion timelines, the population structure between these two species is remarkably different. *V. sieboldii* has seemingly yet to overcome the low genetic diversity associated with the founder effect but is one large population. While populations of *V. dilatatum* have overcome the founder effect and populations are expanding and recovering there is still two distinct populations. Based on what we know from this and the previous two chapters *V. dilatatum* is likely to be the more pervasive invasive species. NJISST has annotated how common and widespread *V. dilatatum* is, while *V. sieboldii* is still uncommon; despite both being considered high threats to natural
areas by displacing native species (New Jersey Invasive Species Strike Team 2017). In chapter two, the New Jersey state data showed that there are more populations of *V. dilatatum* than *V. sieboldii*. Despite being dispersal limited, *V. dilatatum* is populous and populations are recovering more quickly from the founder effect than *V. sieboldii*. However, all of the interpretation for this chapter should be tempered by the fact that the data for this last chapter was a small subsample. Therefore, we likely did not capture all the genetic diversity between the populations and therefore, may not be a complete picture of the population dynamics. To ensure that our preliminary understanding of the population genetic structure holds true, sample sizes should be largely expanded in future analyses.

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Conclusion

The main questions of this dissertation were: are the dispersal “strategies” employed by closely related species comparable and what “strategy” leads to greater success at invading communities? My dissertation focused on three ways of approaching this question by studying dispersal, distribution, and the populations genetics of invasive species. *Viburnum dilatatum* and *Viburnum sieboldii* were used as the model species for this investigation because they are newly invasive species that share similar timelines for invasion. This made it feasible to utilize geospatial and molecular techniques along with empirical and experimental field research to address which dispersal strategy is a bigger threat for species invasion. This dissertation suggests that dispersal and reproductive strategies can be predictive of the ecological processes that influence population composition and genetic structure.

Chapter one demonstrated that differences in nutrition content of fruit led to differences in timing and the species that consumed the fruit which had potential implications for dispersal. *V. sieboldii* fruit was consumed during fall migration, primarily by Gray Catbirds (*Dumetella carolinensis*) and that *V. dilatatum* fruit was consumed in the winter, mainly by American Robins (*Turdus migratorius*). The implications for dispersal were that *V. sieboldii* has a higher probability for long-distance seed dispersal than *V. dilatatum* which may explain why *V. sieboldii* is more widely distributed than *V. dilatatum*. Chapter two illustrated how spatial patterns can detect the underlying reproductive and ecological processes associated with local species distribution. Where *V. sieboldii* most likely relies on clonal spread at distances under three meters but is likely dispersed by birds at distances from eight to twelve meters.
While *V. dilatatum* likely spreads by fruit both at distances under four meters by seedfall and by bird-enhanced seed dispersal at distances between six and thirteen meters. Chapter three suggests that dispersal limitation influences gene flow between locations and that generation time and the potential for novel combinations with other cultivars may influence genetic diversity. We found that *V. sieboldii* had a low $F_{st}$ value, indicating individuals among populations are freely experiencing gene flow. While the $F_{st}$ value for *V. dilatatum* suggest significant population structure. This suggest our observations and hypothesis about dispersal were supported. Tajima’s D suggests that *V. dilatatum* has overcome the founder effect and the population is expanding while *V. sieboldii* is still in a genetic bottleneck despite similar invasion timelines. In the end, I can confidently say that these species “strategies” and suite of traits differ. However, figuring out which species is the more successful invader remains largely unanswered. This is because it depends on what metrics you would use to characterize success. For example, is it more important to be widely dispersed but not as densely populated as is the case with *V. sieboldii* or not as widely dispersed but more densely populated as is the case with *V. dilatatum*? Is asexual reproduction better than sexual reproduction if you are an invasive? Ultimately, context matters (i.e. the habitat, the land use history, and the plant community composition) which is why it is so hard to find a universal invasion theory.

So, what are the larger practical applications for this dissertation and more specifically what does this mean for land managers that find these species in their parks? I believe the approach of quantifying the who, when, why of dispersal in conjunction with the modes of reproduction specific to the species are an important step in assessing a species potential for invasion. Observational data, survey work, mapping distributions to
explore modes of dispersal, define species traits, or reproductive proclivity is probably the most feasible approach for land managers who may be resource limited. It would give them a working baseline knowledge for assessing species and deciding on the best course of action for management. This approach can be applied more broadly for any invasive species but is especially useful for any invasive shrubs that relies on frugivores to disperse their seeds. Many shrubs that are now widespread, successful invasive species in northeast forests like Japanese barberry (*Berberis thunbergii*), multiflora rose (*Rosa multiflora*), privet species (*Ligustrum spp*), honeysuckle species (*Lonicera spp*), wine raspberry (*Rubus phoenicolasius*), European buckthorn (*Rhamnus cathartica*), autumn olive (*Elaeagnus umbellata*), and winged burning bush (*Euonymus alatus*), likely relied on similar ecological and evolutionary processes for spread as did the viburnum species in this study. Plus, there are other non-native species that are not as widespread yet like Photinia species (*Photinia spp*), Japanese angelica tree (*Aralia elata*), and six other non-native viburnum species that could be studied using this framework. I believe the framework for studying invasive species set forth in this dissertation is a viable way forward to quantify, assess, and prioritize newly colonized invasive species.

However, caution should be taken when trying to use a specific system and applying it more broadly, as finding universal traits associated with invasive species is often tricky to pin down. For example, trait-based characterizations such as the number of fruit, fruit/seed size, nutrition content are very useful for making distinctions between the dispersal strategies between these two viburnum species. As are traits-based characterizations for reproductive strategies such as vegetative spread versus solely seed dispersal or generation time. However, trait-based characterizations alone would not
account for species dispersal or potential for novel combinations of cultivated species found in arboretums. These two species illustrate the case that while traits are important they need to be considered in the context of the species natural history. Marrying the natural history component with a trait-based approach would provide a more holistic view on how to deal with invasive species before they become entrenched. This approach would be useful to address both applied and theoretical portions of invasion ecology. Studies such as mine provide supporting evidence for this and also provide a baseline to ask more interesting questions about the system. Indeed, our two species are a model example for this as both are considered successful invaders but the degree to how successful they are and the paths they have taken to get there are seemingly very different.