Genetic, Age, and Spatial Structure to Improve Management of Common Privet (*Ligustrum vulgare*)

THESIS

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Abstract

Invasive plants that spread through natural areas are likely to threaten sensitive and unique habitats. Since it is impossible to kill every individual invasive plant, managers need landscape-scale strategies to direct management efforts along invasion sites and corridors of spread. Therefore, managers need information of how invasive plants spread over time and space with respect to sensitive habitats. *Ligustrum vulgare* L. (common privet) is a woody invasive plant that is spreading to sensitive habitats in eastern North America. To assist in developing efficient management approaches for this species, we studied the spatial genetic structure of *L. vulgare* across the landscape within the 325-acre Wooster Memorial Park (WMP) and in three states, using sequences from two chloroplast DNA (cpDNA) genes. For samples taken within the park, we combined patch age, spatial features, and genetic data to reconstruct invasion history and analyze spatial distribution patterns. We conducted a comprehensive survey in the park, mapping and sampling established stands of *L. vulgare*. Ages of 331 patches in WMP were determined by examining tree-rings. DNA was extracted from samples taken from eight sites in Ohio and two adjacent states along with 313 samples from WMP. Haplotype networks were constructed, and 10 to 17 haplotypes were identified. Haplotype frequencies were geographically different among study sites. Lower genetic diversity was found in the eight Ohio sites compared to sites in New York and Pennsylvania, suggesting an invasion pathway from east to west. In the park, invasion time and three invasion phases were

revealed. A similar invasion time and invasion process was observed for all haplotypes. Initial invasion sites were located and their favorable habitats were identified. New establishment and spatial patterns were described through mapping distributions of patches over time. For spatial analyses, we used nearest neighbor analysis, global Moran's I, and local Gi* statistic, calculated from samples in a rectangular region in the park, to describe changes in spatial clustering of patches during invasion. Haplotype distributions were mapped and analyzed with respect to landscape features. Haplotypes formed a mixed spatial pattern in the park and no genetic pattern associated with landscape features was found. Different spatial distribution patterns by different haplotypes were characterized using spatial analyses of average nearest neighbor and Moran's I. Our study suggested the best time for invasive control and the habitats for detecting early established patches. Identifying spatial distribution pattern of initial patches could help predicting spread of invasive plants. Mapping of clusters of old and young patches may assist in invasive control effort by identifying invasion center and barrier.

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Chapter 1: Introduction

Invasive plant species pose serious impacts on large-scale ecosystems, because they have the ability to degrade environmental quality and reduce native biodiversity (Wilcove *et al.*, 1998; Roux and Wieczorek, 2008). An estimated 5000 invasive plants have naturalized in US, many of which have greatly decreased native vegetation and the associated population of animals, causing a great amount of money in annual invasive control (Pimentel *et al.*, 2005). Among the species of woody shrubs that have become invasive are several species of the *Ligustrum* genus. For example, Hoyos *et al.* (2010) reported that the Glossy privet, *Ligustrum lucidum*, has caused serious forest loss over a large scale and indicated that this invasive species can form large single-species stands and suppress the regeneration of native species. Ranney (2004) reported that Chinese privet (*Ligustrum sinense*) has displaced the native shrub layer in over 2.4 million acres in the Southern US. The focus of the work described here is *L. vulgare*, a species of local and regional concern due to its increasing presence in the landscape.

Currently, a search-and-destroy approach is the common approach for invasive plant management used by land managers when there is no information describing how to find the invasive plant species of interest. Although specific control treatments are available, including chemical and mechanical measures, there is no overall control strategy that is

based on the sufficient understanding of the spread pattern within a landscape context of a specific invasive plant species. Because there is insufficient information about invasive plant origins and dispersal vectors, re-infestation may occur in areas previously managed. Therefore, information on invasion history, spread pattern over time and across landscapes, invasion direction and rate, and source populations are crucial in development of new invasive control strategies that would enable land managers to efficiently focus control efforts, save labor, reduce costs, and increase control effectiveness.

Using a geographic information system (GIS), the spatial distribution of invasive species can now be easily and accurately mapped, monitored, and displayed. Potential spread of invasive species can be predicted, incorporating locations and landscape or climate factors (Jarnevich *et al.* 2010; Chen *et al*. 2007). In addition, many spatial analysis tools have been integrated in GIS for visualizing, describing, and statistically analyzing spatial data, which provides technological support to study invasive species (ESRI 2010).

Dendrochronology is the method of determining the age of perennial plant species based on tree-ring analysis. This method has been used to reconstruct invasion history of invasive plants by age determination to answer questions of how and when an invasive species colonized and spread (Dietz 2002; Troupin *et al.* 2006). Regression of age and basal diameter is also used to estimate age when there is no access of a great number of intersections for tree ring counting (Wangen and Webster 2006; Frapper *et al.* 2003). By

mapping the age of individual plants in GIS, a more comprehensive understanding of invasion pattern can be inferred. The analysis of spatial age structure can be used to estimate direction of spread, rates of patch expansion, and how site conditions affect population development (Wangen and Webster 2006; Frapper *et al.* 2003).

Molecular markers have been used to identify genetic diversity, source populations, introduction history, and pattern of spread of invasive plants, since ecological and observational methods are unable to quantify these measurements. Chloroplast DNA (cpDNA), as an organelle genome with limited genetic modification over time, provides better information about colony establishment than nuclear DNA that undergoes genetic recombination every generation. Chloroplast DNA has been used to develop molecular markers in the studies of landscape genetics, phylogeography, and population genetics. Questions of multiple introductions (Provan *et al*. 2005), invasion origin (Gaskin *et al*. 2005), and historical pathway of invasion and range expansion (Troupin *et al.* 2006) have been answered using cpDNA markers. Although the mutation rate of chloroplast genome is low, it can still provide decent resolution to detect genetic differentiation over large geographic scale (Ward 2006; McCauley *et al*. 2003).

Ligustrum vulgare (Common or European privet) is native to Europe, Africa and Asia, and was introduced to the United States primarily as an ornamental plant for use in the landscape before they escaped into natural ecosystem. *Ligustrum vulgare* can form dense thickets, which are able to suppress the native plant species, and produce

monoculture. Currently, *L. vulgare* is the most widely spread among all *Ligustrum* species in North America (USDA-NRCS 2011).

To better understand the how invasive *L. vulgare* has moved across the landscape and how the infestation developed at one natural area, we conducted research with the following objectives: 1) to combine temporal and spatial information on *L. vulgare* invasion to describe the invasion pattern and history in Wooster Memorial Park (WMP), a 325-acre public park in northeastern Ohio USA; and 2) to describe the structure of genetic variation at different spatial scales by investigating the haplotype diversity using two cpDNA genes. Data were used to provide insight into the historical pathway of spread. In this thesis I will discuss the invasion history and spatial distribution pattern of *L. vulgare* over time and space in Wooster Memorial Park in Chapter 2. In Chapter 3, I will describe analyses of spatial genetic structure of *L. vulgare* over landscape features in the park and genetic diversity among sites across three states. In Chapter 4, I offer conclusions, management suggestions, and recommendations for further study.

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Chapter 2: Temporal and Spatial Pattern of Common Privet (*Ligustrum vulgare*) Invasion in a 325-acre Natural Park

Summary

To develop more efficient invasive plant management strategies, we studied the invasion history and spatial distribution pattern of *Ligustrum vulgare* (privet) by linking age and spatial data. We surveyed, mapped, and sampled established privet stands in the 325-acre Wooster Memorial Park, a public natural area in northeast Ohio. We determined the ages of 331 samples by tree ring analysis, and entered the age and landscape information into a GIS. Age class distributions and numbers of privet patches during invasion revealed the invasion history and three phases during the invasion process in Wooster Memorial Park. The invasion history was described by mapping cohort distribution in each year and formation of new patches. By incorporating cover-type data, we found that old patches forming during the lag phase tended to locate at the edge area of different habitats and open places. Spatial analyses, which are nearest neighbor analysis, global Moran's I, and local Gi* statistic, were performed using samples in a rectangular region in the park. Through these spatial analyses, we identified a dispersed spatial distribution pattern at the early stage of invasion, which later became more clustered. A trend of age spatial clustering during invasion was observed and the clusters were mapped. Although

different cover-types provided different growing conditions, establishment of new patches was not associated with specific cover types. Results of this study affirmed that the best time for invasive control is during the lag phase. Managers should monitor the edge habitats associated with early establishment of *L. vulgare* in order to control early invaders and thereby delay the onset of the expansion phase. Mapping of clusters of old and young patches may assist in invasive control efforts by identifying invasion hot spots and barriers.

Introduction

Invasive plants and management

An estimated 5000 invasive plants have become naturalized in North America, including about 140 exotic trees and shrubs, many of which have greatly impacted native vegetation and the associated population of animals (Pimentel *et al.,* 2005). Many invasive plant species were originally introduced as desirable species by the horticulture industry due to their ornamental or practical value (Reichard and White 2001). Significant financial resources are spent annually to manage invasive plants that have colonized and spread to areas where they interfere with economic activity, transportation, or enjoyment of the outdoors. In natural areas, invasive plants are a particular problem where they displace native species and threaten sensitive habitats that harbor rare or endangered plants. Invasive plants impact not only native plants with which they

compete, but also the many organisms of diverse taxa that depend on particular plants as sources of food, habitat, or protection. For example, invasive shrubs have had negative impacts on nutrition and nesting of birds of conservation concern and can act as an ecological trap for some species (Rodewald 2011).

Spatial distribution and invasion patterns

Because some invasive species can rapidly colonize all kinds of disturbed sites and extend their population, it is very difficult to control invasive plants. By the time most species are recognized as invasive, eradication is virtually impossible. Given the limited resources available for invasive plant control, the best that most managers of natural areas can do is to monitor a limited portion of the landscape with the hope of checking the spread of invasives that might threaten the most vulnerable habitats. This task is especially difficult because of the many unpredictable modes of dispersal of invasive propagules into natural areas, especially those surrounded by significant human activity. In addition, insufficient understanding of spread behavior of invasive plants within a landscape context inhibits the development of efficient control strategies.

The ecological process underlying the possible displacement of native plant species with invasive species is complex. Factors such as landscape characteristics, competitive interactions, topography, and soil properties, can influence the process and consequence of invasions (Tecco *et al*. 2007; Lundgren *et al.* 2004). Landscape features, such as roads

and cover-types can affect invasive plant occurrence, operating as vectors or barriers. Biological attributes of the invasive species interacting with the habitat result in a particular pattern of invasion that might aid managers in making decisions about how and when to focus monitoring or control efforts (Minor and Gardner 2011). Understanding of characteristics of spread of invasive plants at the population and landscape level could reveal the history of plant invasion and allow managers to make predictions about the direction, speed, and consequences of future invasions (Flory and Clay 2006).

Patterns of invasion history can help us understand the time frame over which invasive plants invade and how they spread through a particular landscape. Patch expansion rates, and dispersal facilitations and limitations by site conditions can also be revealed (Dietz 2002; Frappier *et al*. 2003; Lundgren *et al.* 2004). Invasion history can be reconstructed by studying the spatial and temporal patterns during the invasion period. For example, historical aerial photos (Brown and Carter 1998), age determination (Dietz 2002; Frappier *et al*. 2003; Wangen and Webster 2006), and density evaluation (Flory and Clay 2006; Fei *et al.* 2009) have been used for detecting spatial and temporal patterns of invasive plants. Efforts to link spatial and temporal aspects of invasion can provide new perspectives to understand historical process of invasive plants on landscape level. The invasion history over landscape can be reconstructed based on the spatial locations of individual plants at each temporal scale (Wangen and Webster 2006; Frappier *et al*. 2003). Dietz (2002) determined the age of five invasive plant species within patches

using annual ring analysis, and discovered the directional spread preference and distribution pattern via age spatial structure.

Ligustrum vulgare

Ligustrum vulgare (common privet or European privet) is a branched, deciduous or semievergreen shrub, native to Europe, North Africa, and western Asia. It was introduced to North America from Europe in the colonial period as hedge plant (Cothran 2003) and is now naturalized throughout the range. It escaped cultivation and is considered to be invasive mostly in temperate and eastern states and southern Canada (Cothran 2003; USDA-NRCS 2011; Starr *et al.* 2003). *Ligustrum vulgare* was widely planted by homeowners in home landscapes and by state transportation departments along highways. More recently, *L. vulgare* has lost favor to other species due to twig blight anthracnose (*Glomerella cingulata*), which causes leaf yellowing (Dirr 2009). *Ligustrum vulgare* can produce more than 10000 fruits per plant, and fruit number and seed production are not significantly affected by defoliation on flowering branches (Obeso and Grubb 1993). The seed dispersal can be facilitated by berry-eating birds or animals during winter. Although it thrives in full sun and along stream banks, it is tolerant of shade and drought, and can grow in almost any kind of soil (Gratani and Foti 1998; Bailey 1922). It frequently invades riparian habitats and forest edges and can form dense thickets that displace native plant species in natural areas (Weber 2003). The *Ligustrum* genus belongs to the Oleaceae (Olive family) and several other species of *Ligustrum*, such as *L. amurence*, *L.*

japonicum, *L. lucidium*, *L. ovalifolium*, *L. sinense* and *L. quihoui* are also considered as invasive plants in North America (USDA-NRCS 2011; Maddox *et al*. 2010).

In northeastern Ohio, *Ligustrum vulgare* is found in home landscapes and has escaped to some roadsides and waste areas. It is not considered to have reached the level of infestation of other invasive shrubs, such as several species of *Lonicera*. Therefore, we judged that the *L. vulgare* invasion is still in progress, so understanding the history and pattern of the current invasion might provide insight into future spread. In this study, we combined temporal and spatial information of *L. vulgare* invasion using a geographic information system (GIS) to reveal the invasion pattern and history in a natural park in Ohio.

Materials and methods

Study sites and sample collection

Our study site is Wooster Memorial Park (WMP), a 325-acre natural area and public park in Wayne County, Ohio. The park features streams, steep ravines, rich spring flora, and foot trails. It is rich in plant and animal species, including some endangered species, such as *Carex cephaloidea*, and threatened species, such as *C. sprengelii* and *C. retrolexa*. The core area of the park is regrowth maple-oak-hickory forest, while yellow birch and large-

toothed aspen remain following the retreat of the last glacier. Parcels of abandoned farmland in various stages of succession have been added over time.

We mapped and collected basal stem samples from 345 *L. vulgare* plants over the entire area of the park. A crew of four people walking about 10m apart side-to-side surveyed the entire park over a period of 25 days in 2010. A patch of *L. vulgare* was defined as a cluster of *L. vulgare* stems at least 10 m from other *L. vulgare* individuals. If an isolated *L. vulgare* plant was found to be least 10 m from any other plant, it was designated as a nascent patch. The park was revisited in 2011 to confirm that no patches had been missed. In each patch, the stem with the largest basal diameter, which was assumed to be the oldest in the patch, was selected for sampling. We assume that the age of each sampled plant represents the age of the specific stand and that all the patches in the park were sampled and mapped. We measured and recorded the diameter of the main stem at 50cm height. The main stem was sawed off at the soil level and a 4-to-8 cm long core section of the stem base was obtained for age determination (see below). The remaining stump was marked with paint for possible resampling. Site conditions and surrounding vegetation were recorded for all samples. All the samples in WMP were geo-referenced, and data entered into ArcGIS version10 for analysis (ESRI Inc., Redlands, CA).

Age determination

The cut-surfaces of stem-core-samples taken from WMP were sanded with a belt sander to obtain a very smooth surface. This surface was scanned to produce a digital image for annual ring analysis. Annual rings were counted manually and recorded in the program WinDendro (Regent Instrument Inc, Canada). We were able to determine ages of 331 of the 345 *L. vulgare* samples by annual ring counting. Missing samples were due to low quality of some of the digital images of the tree rings. All the age data were combined with GPS coordinate information in an attribute table in ArcGIS v.10. We added the age data in the ArcMap as a GIS data layer and generated a map of age spatial structure.

Statistical analysis of spatial and age data

We generated age spatial structure map based on the age information and locations of each sample. To determine the spatial distribution pattern and habitat preferences of early invasion, we identified the locations of 12 initial colonists in the park as those samples with the highest number of annual rings and occurred during lag phase (Table 1 and Figure 3). We overlaid map layers for putative patch age, cover-type (Shape-file source: Friends of Wooster Memorial Park Volunteer Organization, 2010), and an aerial photo of WMP (Map source: Wayne county Auditor's office, 2004). Record of site conditions and surrounding vegetation from sampling was also referred to. The spatial distributions of *L.*

vulgare during invasion process were described in four years showing the invasion status in those representative years.

Spatial analyses were performed using spatial statistics tool in ArcGIS v.10 to detect and characterize the spatial pattern. Nearest neighbor analysis was performed by Average Nearest Neighbor to test significance of clustering degree of privet patches in the park. Significance in nearest neighbor analysis indicates that the patches are more likely to be spatially close or separated only by chance (Peacock *et al*. 2008). We created data layers, which includes accumulated sample points from the beginning, for each year from 1984 and performed nearest neighbor analysis on each of the data layers. To compute the degree of correlation between patch age and spatial distances during the invasion process, spatial autocorrelation coefficients by each year were recorded using Moran's I on each of the data layers with age values. Hot spot analysis was performed with all the samples using Getis-Ord Gi* statistic (Ord and Getis 1995) in ArcGIS v.10 to detect and locate the high and low age clusters in the park. The Getis-Ord Gi* hot spot analysis can provide information of where the features with similar high or low values spatially cluster in the study area. Z score was used to measure the significance of null hypothesis with 90% confidence level. The non-significant zone by Z score is between -1.65 and 1.65 showing a random spatial pattern. The higher the Z score is, the more intense the clustering for Moran's I or dispersing for nearest neighbor analysis. Because the outer borders of WMP are quite irregular, we selected the largest rectangular area of the park for all the spatial analysis so that edge irregularity would not affect the accuracy of

estimation of spatial pattern and its significance (Fortin *et al.* 2006). The rectangular area included 201 samples from different cover-types representative of the whole park.

Effect of cover-type on age and growth rate

Growth rate (average increase in diameter per year) of *L. vulgare* was calculated by dividing diameter (cm) by age (year) for all the samples that were age determined. We did regression of growth rate and their age of all the samples in GS+ version 5 (Gamma design software, Inc. 2000) to investigate if there is a dynamic trend of growth rate associated with the time of invasion during invasion process. To investigate effects of forest types on growth rate of *L. vulgare*, we calculated the average growth rates of samples located in four different habitats that are hemlock-hardwood upland forest, bottomland forest, hemlock-hardwood slope forest, and evergreen forest. In addition, we compared ages of samples among these four different habitats. To assign samples to habitats, we overlaid cover-type map with sample points that presented in different habitats. All the samples outside the park or at the edge of two habitats, where it is difficult to assign the samples, were excluded, resulting in 310 samples that were successfully categorized to habitats.

Results

Invasion history in Wooster Memorial Park

The pattern of *L. vulgare* patch number and age followed a Gaussian distribution, with a long right tail, indicating low frequencies of the old age classes (Fig. 2). Age distributions were continuous from the four-year-old age class to 23-year-old patches. There were no patches of 3, 24, 26, or 30 to 38 years old. The oldest patch or individual plant was 39 years old, suggesting that the invasion of *L. vulgare* into this park started at least 39 years ago.

We observed three phases during 39 years of invasion of *L. vulgare* in Wooster Memorial Park (Fig. 3). Following the initial invasion in 1972 there was a 19-yr (1972-1990) lag phase during which 12 initial patches or individuals started as colonists. A rapid increase in patch number started from around 1991 and continued to 2006. The average increase of patches was approximate 19 per year and the maximum increase was 35 per year, which occurred in 1994. During the expansion phase, a total of 310 patches or individuals established, which is about 94% of all the patches we aged. The rate of new patch establishment decelerated suddenly beginning in 2007.

A map of spatial structure by age class was generated using ArcGIS v.10 by combining the age information with coordinates of each sample overlaying on layer of map

boundary (Fig.4). Privet patches with different age classes were spatially mixed in the age spatial distribution map. In other words, there was no evidence of an invasion front for specific direction of movement of the invasion in the park from observation. We analyzed the locations of the initial patches from the lag phase by overlaying the age layer, cover-type layer, and aerial photo, and referring to the record from sampling. We found that the colonization of the oldest age class (the 21-39 year-old) appeared to be associated with edge habitats, where animals are more active (Table 1). Open sites were also preferred, possibly due to the plant's preference for light and higher survival at open spaces. The oldest patch was found to be at the edge area of the woods, adjacent to a farm. Although some citations suggest that *L. vulgare* prefers bottomland habitats (Olson and Cholewa 2009; Weber 2003; Webster *et al*. 2006), most of the oldest patches in WMP were located in upland forest sites while only two are found on the bottomland (Table 1).

Spatial distribution pattern over time

To better understand the invasion process from a spatial perspective, we compared spatial distribution maps of privet patches in each year during invasion period (Fig. 5). Only 4 representative maps in four years were demonstrated here. From these observations, the privet invasion sites in the park were highly dispersed and exhibited a large geographic scale pattern during the lag phase and the first part of the expansion phase (Fig.5. year 1991 and 1996).

To further study the clustering status of *L. vulgare* patches on statistic level and how it changed during the invasion process, we performed the nearest neighbor analysis using coordinates of all the sample data by each year. The Z-score values larger than 1.65 suggest significant dispersing pattern of *L. vulgare* patches before year 1995 except for the year 1991 during the early invasion (Fig. 6). A trend of spatial pattern from dispersing towards clustering was observed over time. However, *L. vulgare* distributed randomly in the studied area in most of the years during the invasion history.

We also used Moran's I as a means of evaluating the age spatial patterns, which can be clustered, dispersed and random, based on age and geographic locations of *L. vulgare* patches. In this case, we observed a spatial clustering trend of age distribution starting around 1998 following a fluctuation within the zone as random pattern (Fig. 7). The Z score larger than 1.65 indicate significant clustering of patches from 2003. The spatial clustering trend started relatively late (around 1998) during the invasion, which may possibly indicate the formation of low value clusters, that is, the occurrence of young-age privet clusters.

To identify the clusters of young and old *L. vulgare* patches, a map from Getis-Ord Gi* hot spot analysis was generated (Fig. 8). Two old patch and two young patch clusters were discovered. The bigger cluster of old patches in the northeastern upland forest was found to be in a younger woods, which are thought to allow a higher density of invasive

plants compared to older woods (Flory and Clay 2006). The larger young privet cluster was located on the upland forest, which is partially isolated by the farmland in the west and grassland in the south. Furthermore, the eastern and northern sides of this area were slope habitats connected with bottomland forest. Therefore, although some old patches including a initial patch were found in this area, a low number of *L. vulgare* plants established during the early years due possibly to the lack of source population and the isolation of the site. Based on this assumption, an east-to-west slope appeared to inhibit the spread of *L. vulgare*. It is also possible that a great number of well-established invasive plant species in this area (mostly *Lonicera* spp.) prevented privet establishment at this site by occupying the understory of the woods.

Ages and growth rates in four different habitats

Growth rates were examined against the age of each sample, generating a slightly negative slope of the regression line, which is not significantly different from zero. Therefore, we did not find any relationship between growth rate and age of *L. vulgare*. We compared age and growth rate among four cover-types. The growth rate of *L. vulgare* in the bottomland forest is significant higher than the growth rate of privet growing in the upland forest (Fig.9). However, there is no significant difference of average ages of privet among four cover-types.

Discussion

Invasion history in Wooster Memorial Park

Because of their long juvenile (pre-reproductive) period, woody perennial invasive species usually have a long lag phase. This is the first phase of an invasion, during which expansion is delayed, likely due to plant adaptation and competition (Petit *et al*. 2004). Controlling invasives at this stage can help stop further spread through various methods of seed dispersal. The discovery of favorable habitats for initial patches during lag phase can assist early detection of *L. vulgare* or even other invasive plants. However, the lag phase with small populations is difficult to detect, so the control efforts are usually initiated during the second phase after invasion becomes apparent, which makes predicting the exponential growth rate of invasion very important for invasive management (Radosevich *et al.* 2003). The sudden deceleration in patch initiation after 2006 may have resulted from saturation of favorable habitats, which may lead to lower germination or establishment of new *L. vulgare* plants. In addition, adjacent patches can merge to form bigger patches; therefore, at some point of patch density, new colonists located in or adjacent to old patches cannot be distinguished. The saturation phase in this study, when the rate of new patch formation declined, is likely an artifact of our ability to distinguish new plant establishment and probably does not indicate a decline in population dynamics. *Ligustrum vulgare* density could have increased at a considerable rate after year 2006, but the geographical extent of new patch development in the park

slowed at this point.

Spatial distribution pattern over time

Using nearest neighbor analysis, we observed a trend in the spatial distribution pattern from dispersed to clustered. However, the distribution pattern was random in most of the years (Fig. 6). By observing the spatial distribution map in each year, we also found large geographic extent of patch establishment during the entire invasion process (Fig 5). The initial patches that were highly dispersed over the park may contribute to the spatial pattern at the late stage of the invasion. These initial patches may have served as satellite populations facilitating local recruitment in the later part of the expansion phase and the saturation phase. Satellite populations are generally easily overlooked in invasive management, although they play important roles as sources of population in invasion expansion (Ghersa *et al.* 2000; Radosevich *et al*. 2003).

We successfully demonstrated how *L. vulgare* spatially distributed from 1972 to 2010 in WMP by linking the ages and the corresponding locations of the patches. There was no clear invasion front of *L. vulgare* population in WMP. No cluster circling of the oldest starting patch was observed. The complicated and larger landscape environment most likely provided varying habitat conditions, which may have influenced germination, growth, and survival (Tecco *et al.* 2007). The mosaic of environmental driven factors may have greatly increased the noise of invasion pattern. In addition, the highly dispersed

initial satellite patches, which may have resulted from multiple randomly dispersions from the source population, could have functioned as source patches facilitating the invasion pattern that we observed. The mapping of the clusters of younger and older patches provided evidence of possible invasion center and invasion barrier, both of which were significant in invasive management. The big cluster of old patches analyzed in part of the park area may still suggest a hot spot of invasion in the entire park.

Our results showed that the growth rate of privet in bottomland forest was significantly higher than that in upland forest. Moisture and light availability of bottomland forest may provide a good growing condition for privet in WMP. However, establishment of *L. vulgare* in WMP may not be significantly affected by cover-types. Although the clustering of age spatial pattern from analysis of Moran's I suggested a preference of habitats, the clustering may not be associated with cover-types. More information of landscape features may be needed to further understand their associations with early or late establishments.

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Age (Number of samples)	Locations
39 (1), 28 (1), 21 (1)	Farm-upland forest
29 (1)	Trail-stream-slope forest
28 (1), 22 (1)	Upland-slope forest
27 (1)	Grassland-evergreen forest
25 (1), 23 (1)	Upland forest
22 (1)	Bottomland forest (open area)
21 (1)	Stream-bottomland forest
21 (1)	Edge of the park (upland forest)

Table 1. The locations of the oldest patches during lag phase (1971-1990). Dashes represent the edge area of two or three different connected habitats.

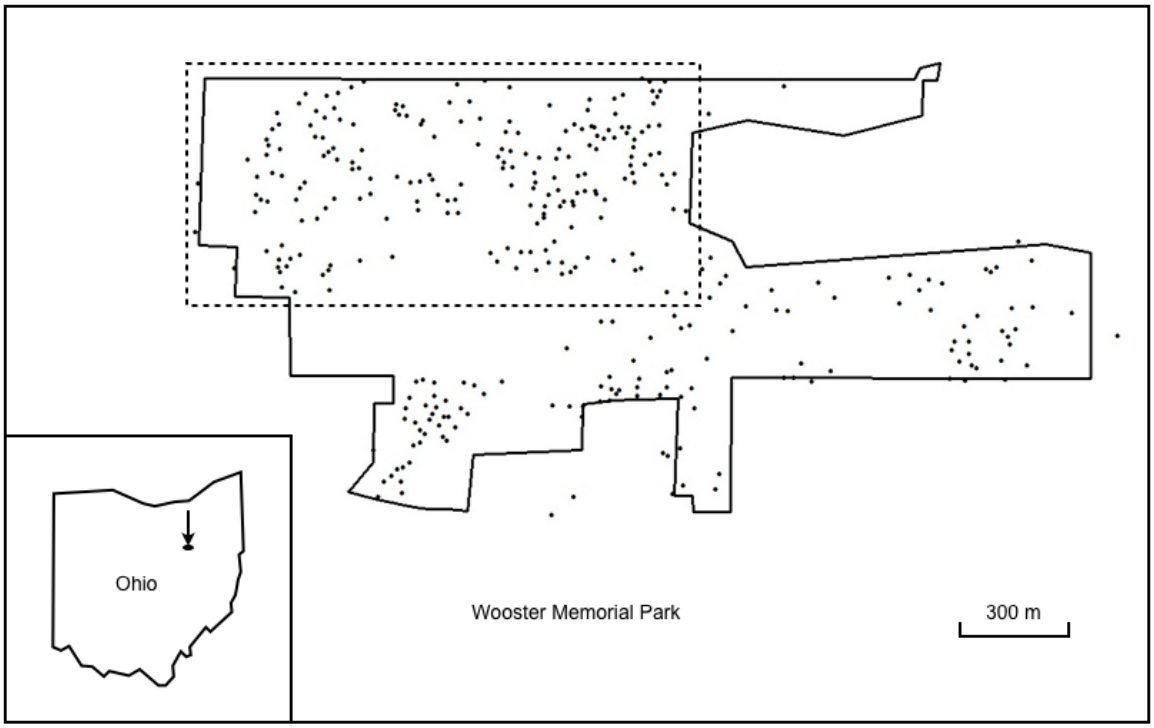

Figure 1. Geographic locations of sampling site and map. All the *L. vulgare* samples are represented as a dot in the park map. The rectangular region for spatial analysis is indicated by dashed line.

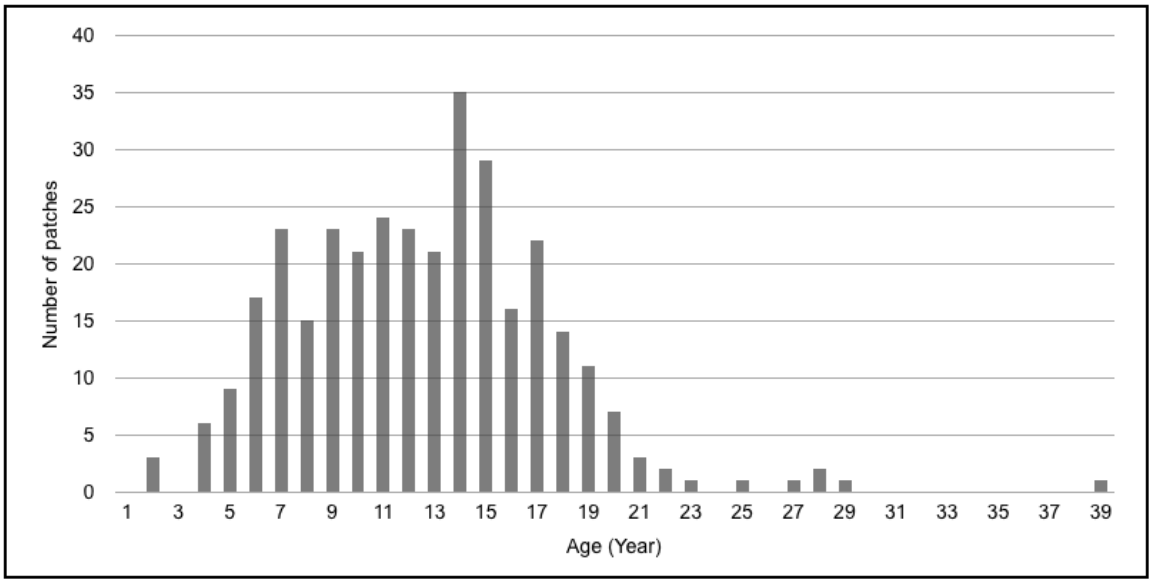

Figure 2. Age distribution of 331 samples. The number of *L. vulgare* patches in relation to patch age for all samples obtained at Wooster Memorial Park.

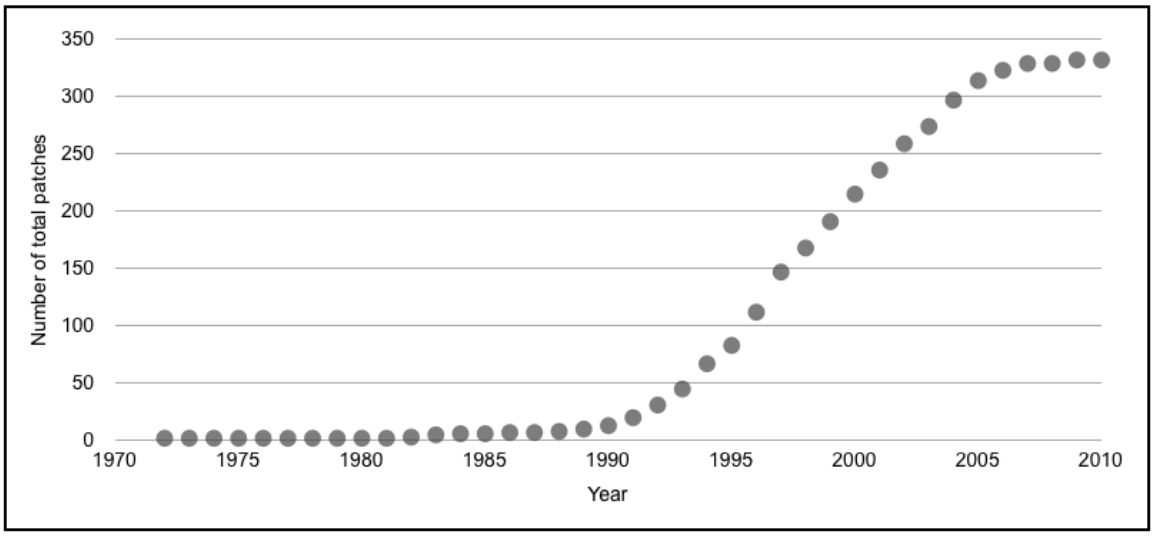

Figure 3. Total number of *L. vulgare* patches by each year.

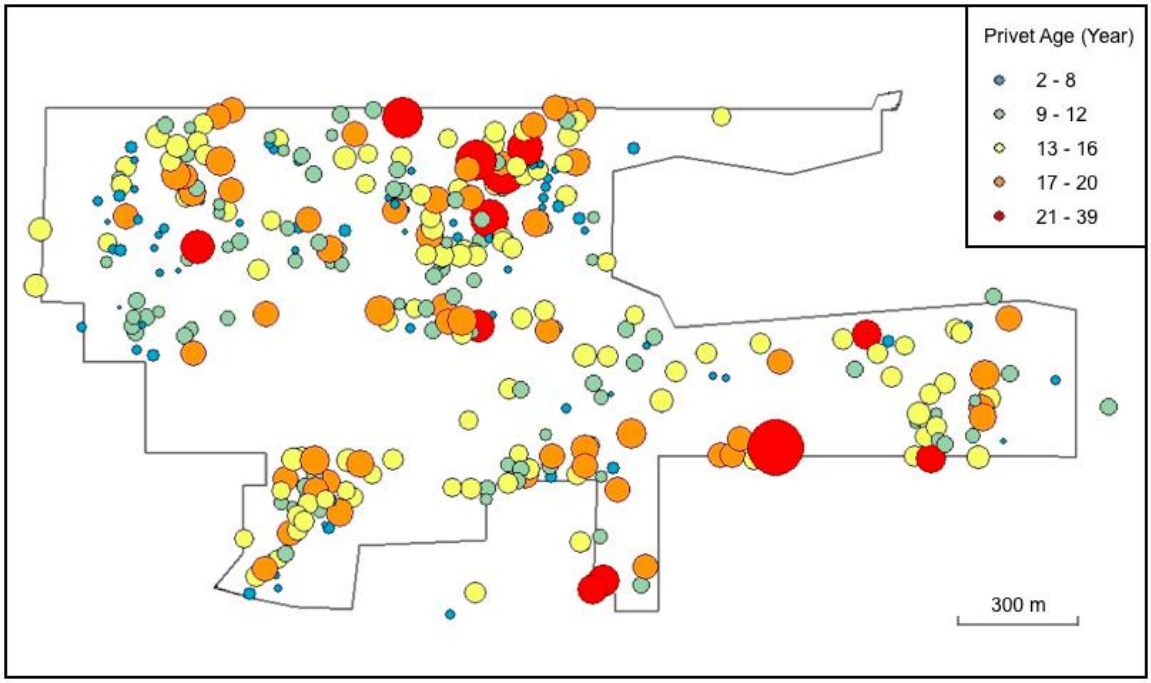

Figure 4. Age spatial distribution across park landscape. The colors representing different age classes are indicated in the legend. The sizes of the circle also represent the ages of privet patches. The bigger the circles the older the patches are. The age class division interval and class number were generated by the program default setting. The oldest sample of 39 year-old is at the southeastern of the park showed by the largest red circle.

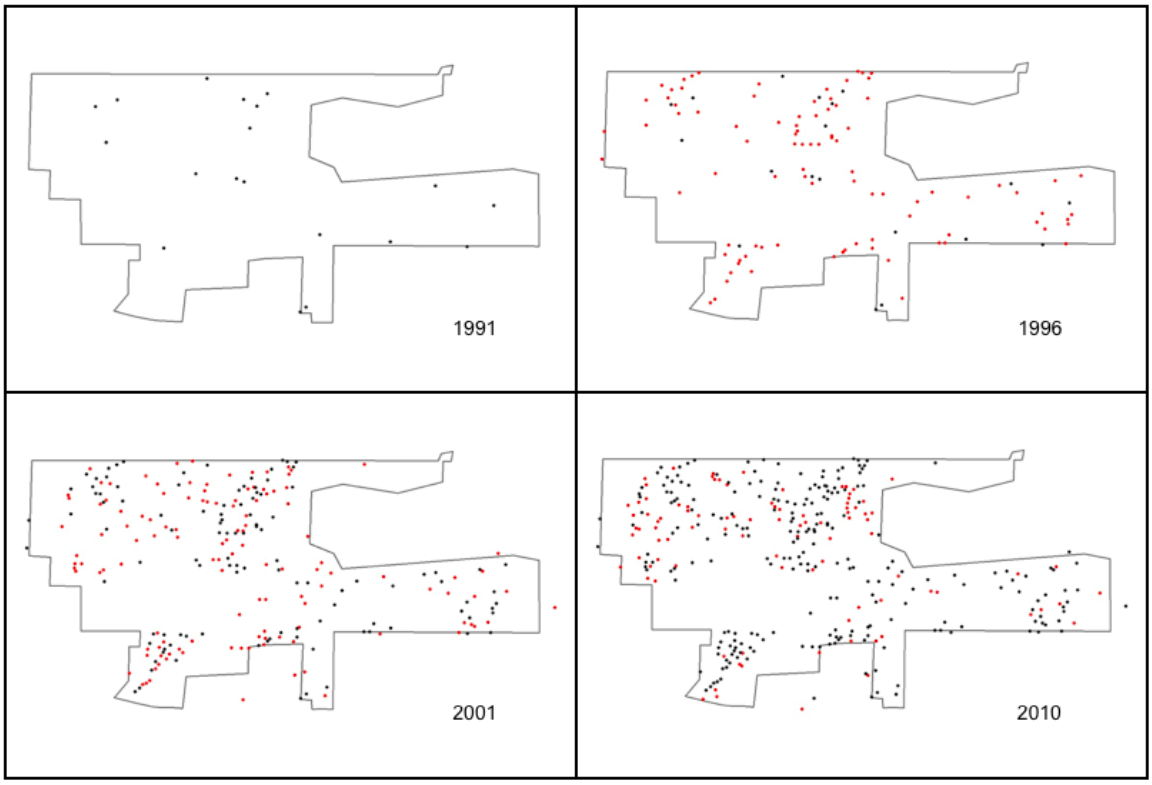

Figure 5. Spatial distribution of privet patches in four representative years. Year 1991 shows the first year of expansion phase. Year 1996 shows the early session of expansion phase. Year 2001 shows the late session of expansion phase. And year 2010 presents all the patches we sampled.

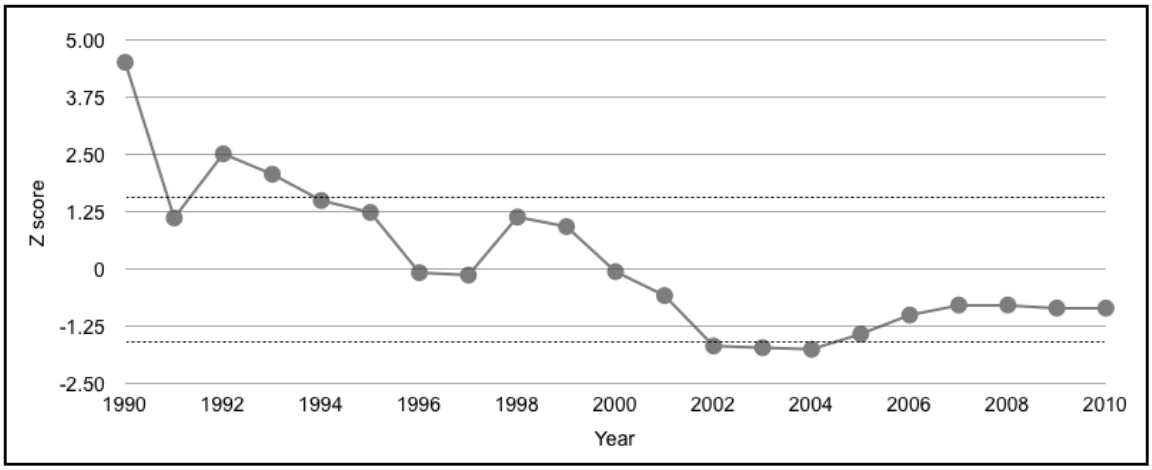

Figure 6. Clustering status during invasion by nearest neighbor analysis. The spatial clustering patterns in each year in the square area are represented by normal standardized scores (Z score values), which indicate the intensity of clustering. Area between the two dashed lines (-1.65~1.65) indicates a random pattern of spatial clustering. Positive significance (z >1.65) indicates dispersed pattern and negative significance (z <-1.65) indicates clustered pattern.

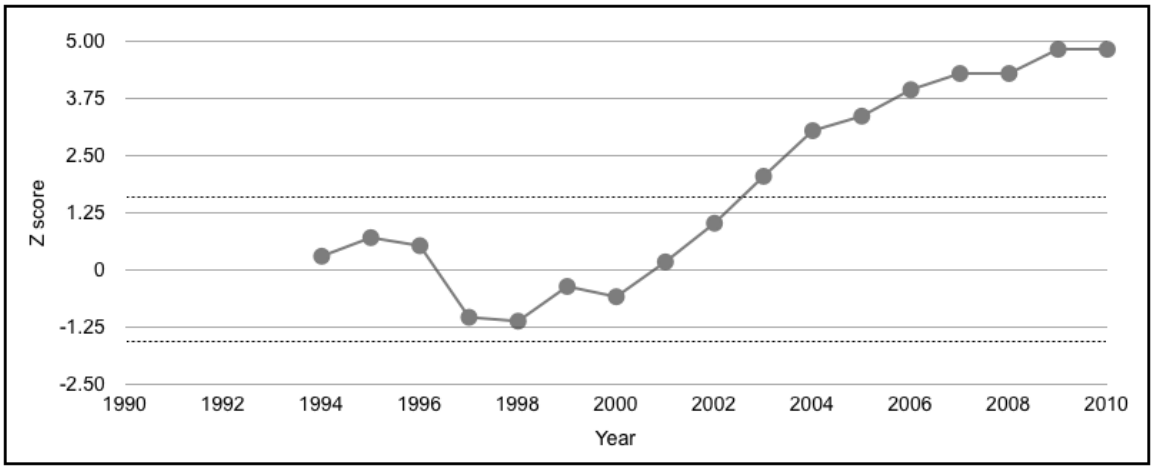

Figure 7. Age spatial autocorrelation in each year by global Moran's I. Clustering pattern of similar age values are represented by Z-score value recorded in each invasion year. A minimum of 30 samples starting from 1994 were required to obtain an accurate estimation. Non-significant area (-1.65~1.65) indicating the random pattern of age spatial correlation is between the dashed lines. Clustered pattern is measured by Z-score over 1.65 while dispersed pattern is represented by Z-scores less than -1.65.

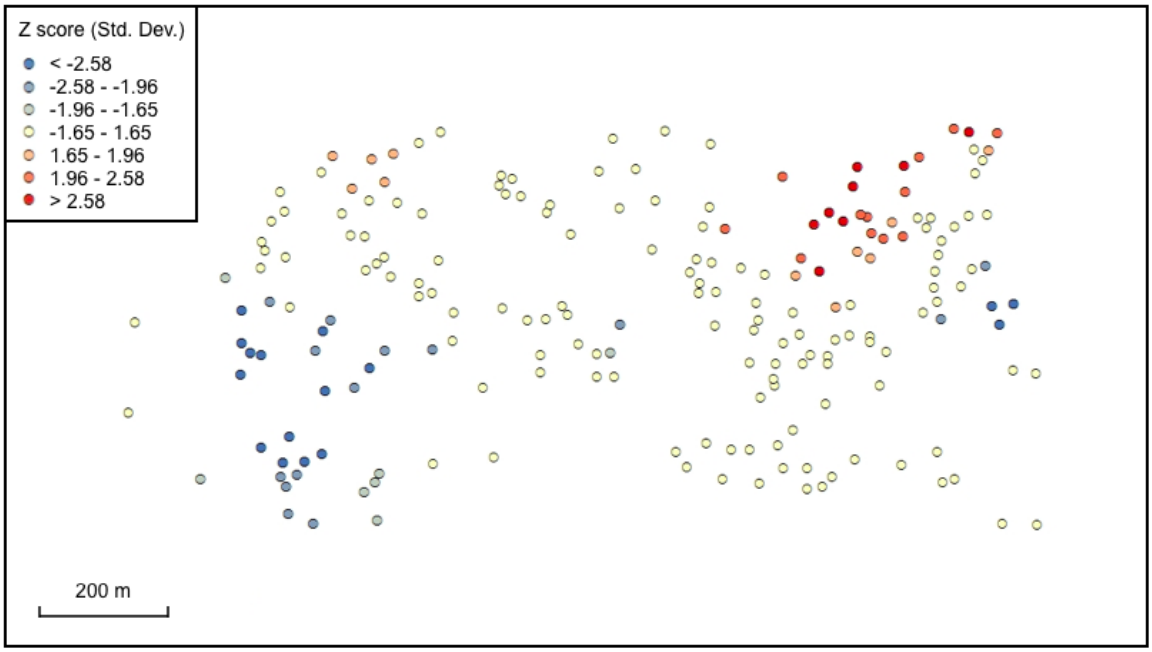

Figure 8. Map of clusters of younger and older patches. Map generated according to Getis-Ord Gi* statistic indicates high and low age clusters in the square area in 2010. The local Gi* statistic is represented by a Z score. The Z score values indicate the intensity of the clustering of high or low value. In the map, points with blue colors are young privet patch that form the low value clusters. Red-color points represent plants in old patch clusters; yellow points are non-significant ones.

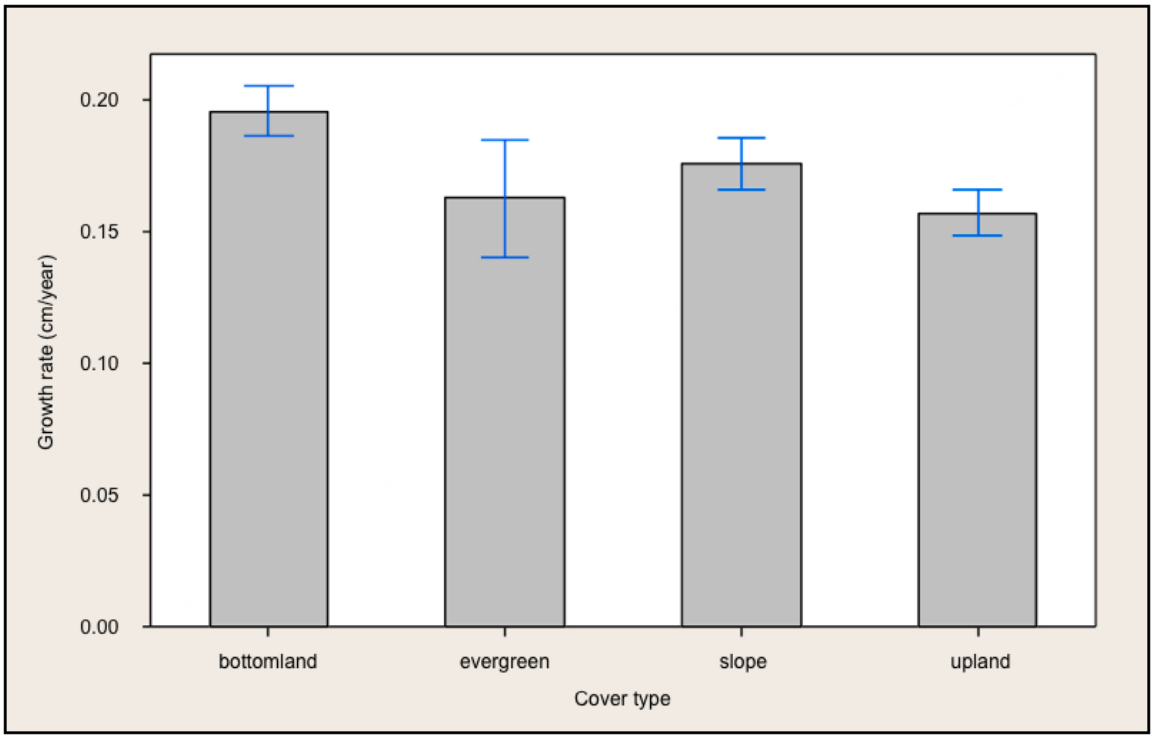

Figure 9. The growth rates of *L. vulgare* among four cover-types. Bars of standard error from mean are shown. The growth rate of privet in bottomland forest is significant higher than the growth rate in upland forest

Chapter 3: Chloroplast DNA markers reveal invasion history of *Ligustrum vulgare*

Summary

Ligustrum vulgare (Common privet or European privet) is a woody invasive shrub that can threaten sensitive and unique habitats in natural areas of the eastern United States. *Ligustrum vulgare* is one of the most widely spread species in the *Ligustrum* genus. To develop landscape level strategies for management of privet, we studied the haplotype spatial distribution pattern of privet across three states and inside Wooster Memorial Park. Two chloroplast DNA (cpDNA) genes were used to characterize haplotypes in eight sites from three states and from samples of different *Ligustrum* species in commercial nurseries. Haplotype networks were constructed and 10 to 17 haplotypes were identified. Nursery species were highly genetically different among each other and from samples in study sites. Haplotype frequencies were geographically different among study sites. Lower genetic diversity in sites of Ohio compared to two eastern states was detected, suggesting an invasion pathway from east to west. We comprehensively surveyed the 325-acre Wooster Memorial Park, mapping, sampling and aging established *L. vulgare* stands. Age, spatial data, and genetic information were linked to study the invasion history and spatial distribution pattern of this species in Wooster Memorial Park. A similar invasion period and invasion progress was observed for different haplotypes.

We found no association of particular haplotypes with landscape features. Different spatial distribution patterns by different haplotypes were characterized. Understanding of haplotype pattern in the park and the possible source populations will facilitate developing efficient management strategies.

Introduction

Ligustrum vulgare (common privet or European privet) is a branched, deciduous or semi-evergreen shrub. It is native to Europe, North Africa, and western Asia, and was introduced from Europe in the colonial period as hedge plant (Cothran 2003). *Ligustrum vulgare* first became naturalized in many eastern coastal states and now is naturalized throughout most of the United States, and is considered to be invasive in temperate and eastern states and Southern Canada (Cothran 2003; USDA-NRCS 2011; Starr *et al.* 2003). It was first offered in an American nursery in early 19th century and many varieties were sold as recorded in early nurseries (Cothran 2003). The *Ligustrum* genus belongs to the Oleaceae (Olive family) and several other species of *Ligustrum*, such as *L. amurence, L. japonicum, L. lucidium, L. ovalifolium, L. sinense* and *L. quihoui* are also considered as invasive plants in North America (USDA-NRCS 2011; Maddox *et al*. 2010).

Ligustrum vulgare was widely planted as a hedge in home landscapes and along highways, but has lost favor to other species due to development of more desirable

species and to twig blight anthracnose (*Glomerella cingulata*), which causes leaf yellowing (Dirr 2009). *Ligustrum vulgare* produces creamy-white flowers with strong aroma in late May and June. The mechanism of pollination is unknown and is suspected to be insect-pollination due to the fragrant white flowers (Starr *et al.* 2003). It produces abundant black berry-like fruits that ripen in autumn, persist into spring, and are dispersed by birds and animals over the winter. *Ligustrum vulgare* can produce more than 10,000 fruits per plant, with 1 to 4 seeds per fruit (Obeso and Grubb 1993). It is tolerant of shade and drought, and can grow in almost any kind of soil (Gratani and Foti 1998; Bailey 1922). Where it escapes from cultivation, *L. vulgare* commonly invades into riparian habitats and forest edges and can form dense thickets that displace native plant species in natural areas (Weber 2003). Various species of *Ligustrum* have invaded natural areas in eastern North America, where they have the ability to spread and form nearly continuous thickets in the understory of forests (Batcher 2000). *Ligustrum vulgare* can reach heights of 3-5 m and grow in a variety of habitats, including old fields, floodplains, mature and disturbed woodlands and woodland borders (Batcher 2000). They tolerate a wide range of soil types and moisture regimes, and prefer sun to part shade (Bailey & Bailey 1976). In addition to reproduction by seeds, established plants can regenerate from root and stump sprouts, forming dense patches.

Like other woody invasives, *L. vulgare* plants are often found in patches dispersed across a complex landscape, among a tangle of native and sometimes protected species, where they are difficult to detect, especially in early stages when control would be most

effective (Dietz 2002). Natural area managers often undertake control measures with little information about sources of invasive genotypes or the pathways of dispersal that could be used to develop landscape-scale strategies that take into account the source and direction of infestation. With better information about the age structure of an invasive plant infestation, genetic relationships within and outside of the management area, and the spatial distribution across the landscape, managers could develop control strategies that are more efficient and that reduce the potential for re-infestation from the same or other source populations.

Chloroplast DNA (cpDNA) of plants has been widely employed to investigate interspecies and intraspecies evolutionary history (Petit and Vendramin 2007). As an organelle genome, the chloroplast genome is transferred to offspring only along maternal lines in most plant species without recombination, which provides a non-mendelian perspective to understand population structure. In addition, spatial distribution of new invasive colonies, which established only by seeds, can be better interpreted by studying maternally inherited genes. The cpDNA genome also provides an appropriate resolution to reveal the historic origin because of its relatively slow rates of nucleotide substitution (Hartl and Clark 1997). Those characteristics make cpDNA an ideal marker for phylogeographic studies.

Differentiated pollen or seed dispersal patterns are important factors shaping population structure. New patches are established by seeds; therefore, the study of maternally

inherited genes can be used to understand the spatial distribution of plants over time (Petit and Vendramin 2007). A highly structured chloroplast genome signifies limited seed dispersal, while a low level of genetic divergence among cpDNA markers indicates more extensive seed transfer (Loo and Burg 2004).

Introduction history is an important factor influencing the genetic diversity in invasive populations. Founder events during introduction cause a loss of genetic variation in invasive species compared to their source population (Dlugosch and Parker 2008). However, multiple introductions can result in similar or higher genetic diversity due to hybridization among introduced genetic variants (Williams et al. 2005).

Geographic patterns of evolution within a species can be understood from haplotype distribution patterns. The ancestral cpDNA haplotype can be inferred as the most abundant haplotype, and its migration route can be identified according to geographic changes in haplotype number and diversity (Loo and Burg 2004; Bain and Golden 2005). For example, during range expansion of *Vriesea gigantean*, haplotype diversity of cpDNA decreased and the ancestral haplotype became more dominant (Palma-Silva et al. 2009). Local mutations of cpDNA possibly due to adaptation have also been observed, resulting in higher haplotype diversity and greater numbers of haplotypes compared to original populations (Bain and Golden 2005).

We conducted research to better understand how invasive *L. vulgare* has dispersed across the landscape and how the infestation developed at one natural area in particular. We studied the structure of genetic variation at different spatial scales by investigating the haplotype diversity using three chloroplast DNA markers (Demesure *et al.* 1995). We examined genetic variation and spatial genetic structure of this species across three states using the same markers to provide insight into the historical pathway of spread. Landscape features and age information were incorporated with haplotype diversity to describe the invasion history and distribution patterns of privet in Wooster Memorial Park (WMP), a 325-acre public park in northeastern Ohio USA. In order to conduct these investigations, genetic and age spatial analyses were performed with respect to patches of *L. vulgare* associated with different haplotypes.

Materials and methods

Study sites and Sample collection

Our main study site, Wooster Memorial Park (WMP), is located in Wayne County, Ohio, with streams, steep ravines, rich spring flora, and foot trails. The park is rich in plant and animal species, including several endangered and threatened species. The central area of the park is regrowth maple-oak-hickory forest, including some remnant stands of yellow birch and large-toothed aspen. The park was established up over time from parcels of abandoned farmland in various stages of succession.

Over the entire area of WMP, we collected samples from 345 privet plants. Each sampled privet plant was selected from a single patch and was selected to represent the oldest (largest basal diameter) individual *L. vulgare* plant in the patch. We assume that the age of the each sampled plant represents the age of the specific patch and we attempted to sample and map all patches in the park, as described previously (Chapter 2). Isolated individual *L. vulgare* plants, separated from others by at least 10 m, were also sampled. We obtained leaf samples for DNA extraction, by selecting 5 to 8 fully formed, disease-free leaves on an individual plant. The main stem was sawed at soil level and a 4-8-cm core section of the stem base was taken for age determination by counting of annual rings. Patch density and surrounding vegetation were recorded for each sample. All the samples in WMP were geo-referenced, and data entered into ArcGIS-10 for analysis.

In addition to samples from WMP, we took leaf samples of *L. vulgare* colonizing five other sites in Ohio and one site each from New York and Pennsylvania (Fig. 10, Table 2). The locations of the sites outside of WMP represented privet populations from northeast to mid-southwest (Fig. 10). At each site, we took leaf samples from 14 to 24 privet plants along trails and in the woods, with broad coverage at each site. Individual sampled privet plants that were at least 10 meters apart were selected. As above, 5-8 fresh and disease-free leaves were collected per plant and stored in a minus 80C freezer.

Chloroplast (cp) DNA marker characterization and genetic analysis

Total DNA was extracted from frozen leaf tissues following the microprep protocol (Fulton *et al.* 1995). Two chloroplast DNA markers, trnS and trnH, (Demesure *et al.* 1995) were identified by single-nucleotide polymorphisms and insertion/deletions using 16 individuals randomly taken within and around WMP. We designed new primers at these regions and used them as markers to identify haplotypes in different local populations. PCR parameters were: 4min at 94 ℃, followed by 30 cycles of 20s at 92 ℃, 20s at 54 ℃, and 1m at 72 ℃, and a final elongation for 10min at 72 ℃. Sequences in both forward and reverse directions were generated by Functional Biosciences, Inc., Madison, WI. Chloroplast DNA sequences were aligned and edited in Bioedit version 7.09 (Hall 1999), resulting in one 679bp sequence (trnS) and one 725bp sequence (trnH). To explore genetic structure and invasive pathway on larger scale, we sequenced samples from eight local populations acorss Ohio, New York, and Pennsylvania using these two sets of primers (Table 2). Since there is no recombination in the chloroplast genome, we connected the trnS sequence and the trnH region to generate a combined marker of 1404bp to provide a higher resolution for detecting variation geographically. In order to describe the spatial distribution pattern of haplotypes in WMP, we successfully sequenced 634bp and 685bp of trnS and trnH regions with 316 privet samples in WMP. A combined marker of 1319bp length was employed for all the samples within WMP and the haplotype information was put into ArcGIS v.10 for further spatial analysis.

Haplotype networks were generated at trnS and trnH using the statistical parsimony method implemented in TCS version 1.21 (Clement et al. 2000). A haplotype network was also constructed using the combined marker with reticulations resolved according to the predictions of coalescence theory (Crandall and Templeton 1993). To better understand the genetic variation in *L. vulgare*, we included samples from nine known *Ligustrum* species or cultivars from a commercial nursery in the haplotype network construction using the three markers, together with the samples from all the study sites. Percentage of samples with different haplotypes was calculated at each study site to describe the haplotype frequencies at different geographic regions.

Genetic diversity in each site and state, and genetic differentiation among Ohio sites and across three states were analyzed using the combined marker that has highest number of variable sites in Dnasp version 5.10 (Librado and Rozas 2010). Genetic diversity was measured by the number of haplotypes, the haplotype diversity (Nei 1987), the number of polymorphic sites and the average number of nucleotide differences (Tajima 1983). Genetic differentiation among study sites in Ohio and across three states was analyzed by statistical test of Hs and Hst (Hudson *et al*. 1992). Probability was obtained by permutation test with 1000 replicates. In analyses of genetic diversity and genetic differentiation, Ohio population was represented by samples in all six sites.

Age determination

The cut surfaces of stem core samples taken from WMP were sanded to obtain a very smooth surface. This surface was scanned to produce a digital image for tree ring analysis. Tree rings were counted manually and recorded in the program WinDendro. We were able to determine ages of 331 of the 345 privet samples by tree ring analysis. Missing samples were due to low quality of some of the digital images of intersections. All the age data were combined with GPS coordinate information in an attribute table in ArcGIS version 10 (ESRI 2010). We added the age data in the ArcMap as a layer and generated a map of age structure.

Spatial analyses with age data and genetic diversity

In ArcGIS, we overlaid a cover-type layer (Shape-file source: Friends of Wooster Memorial Park Volunteer Organization, 2010) with haplotype distribution maps each year during the invasion period in order to examine effects on genetic spatial structure by landscape feature. The cover-types were categorized by land use and forest type into: bottomland forest, hemlock-hardwood upland forest, hemlock-hardwood slope forest, evergreen forest, multi-use area, grassland habitat, and oil field. The Average Nearest Neighbor function for spatial statistics in ArcGIS version 10 was used to describe how the studied samples were spatially distributed with respect to whether they were geographically clustered, random, or dispersed. For this analysis, the null hypothesis was

that the points were randomly distributed. The significance of the test was measured by Z-score and p-value, from which spatial structure was suggested (ERIS 2010). Spatial autocorrelation, based on the sample locations and their age information, was analyzed using the Moran's I function in ArcGIS v.10 to evaluate the spatial distribution pattern by age, with respect to whether the samples with similar age were spatially clustered. In the spatial analyses, both nearest neighbor analysis and Global Moran's I, we used the 90% confidence level to evaluate the null hypothesis. To decrease possible edge effects due to the irregular shape of the park boundary, we selected the largest possible rectangular area, which included 219 samples to perform the spatial analyses.

Results

cpDNA diversity

There were 16 variable sites of 679bp region amplified by trnS; there were 22 variable sites of 725bp sequences generated by trnH and 38 variable sites of combined 1404bp sequences. Three haplotype networks were constructed based on the three cpDNA markers, trnS and trnH, and the combined marker (Fig.11). Ten haplotypes were identified at cpDNA marker trnH (Fig. 11A H1-H10) and 1l haplotypes were identified at trnS (Fig. 11B H1-H11), while 16 haplotypes were identified at the combined cpDNA maker (Fig. 11C H1-H16). There were two dominant haplotypes at trnH, which were found in all the study sites and the other was found in seven sites. Three dominant

haplotypes at trnS were found in all the eight study sites. The combined cpDNA marker also produced three dominant haplotypes, which were found in all the study sites. There were high numbers of mutation events between H2 and H3 at trnH and between H3 and H4 at trnS. Haplotypes found only in the nursery species and cultivars were indicated in all of the haplotype networks (H6-H10 in haplotype network of trnH and H5-H9 in haplotype network of trnS and H12-H16 in haplotype network of the combined cpDNA marker).

Most of the species and cultivars in nurseries were highly genetically different from the individual *L. vulgare* plants sampled in natural areas. There were also high levels of genetic variation between different *Ligustrum* species and cultivars from the nursery. At trnS nine privet species and cultivars contain seven haplotypes; at trnH and combined cpDNA marker, eight species contain seven haplotypes. Five (five by each cpDNA markers) of those seven haplotypes were only found within the nursery (Table 3 and Table 4). The high genetic variations are consistent with the fact that horticultural varieties are developed from broad scale selection and breeding that may involve interspecific hybridization.

Geographic distributions of haplotypes across states

We generated haplotype distribution maps showing the percentage of samples with different haplotypes at each cpDNA marker. Haplotype distribution maps suggested that

frequencies of haplotypes varied geographically (Fig.12). Genetic diversity within Ohio population is low with an average of 2 haplotypes at trnH and 2.3 haplotypes at trnS. At both cpDNA regions, New York and Pennsylvania populations contained more haplotypes than any of the Ohio sites except for RR. The reduction in haplotype number from eastern sites to the Ohio sites suggested a founder event. This loss of haplotype number was observed in five Ohio regions at trnS cpDNA region and in all six Ohio populations at trnH cpDNA region, compared to New York and Pennsylvania populations. Genetic diversity in each site and in each state was measured and demonstrated in Table 5. By analysis of Hs and Hst, no significant genetic differentiation was discovered among six study sites in Ohio. However, genetic differentiation was found to be significant among Ohio, New York and Pennsylvania population with P value less than 0.001.

Spatial genetic and age analysis in Wooster Memorial Park

We looked at the haplotype diversity and distributions in individual patch level on the scale of the 325-acre natural area at WMP. 316 samples in WMP were characterized with three cpDNA markers, generating three (trnS), two (trnH) and six (Combined marker) haplotypes separately. No private haplotype were found compared with the haplotype networks we constructed. Only 4 samples carried H4 to H6 at combined marker and the samples carrying H1 to H3 at combined marker carried H1 to H3 at trnS.

To reveal the invasion period of privet carrying different haplotypes, we calculated the number of patches in each age class by haplotype at each marker (Fig.13). The oldest sample, 39 years old, was found to carry H3 at trnS, H2 at trnH, and H3 at the combined marker. After the oldest patch was established in year 1972, samples with the same haplotype did not occurr until 1988 (trnS and Combined marker) and 1989 (trnH). At the combined marker, H4 occurred in 1994 and 1995; H5 established in 1992 and H6 established in 2004. Although the age distributions of privet with different haplotypes were dissimilar, there was no sign of an early or late invasion of a specific haplotype (Fig. 13). Moreover, the average ages for the various haplotypes were not significantly different. The growth curves indicating the total number of privet by each year showed three phases in the same time period among different haplotypes and same as the time period by all samples from previous study (Fig. 14). This suggests that *L. vulgare* with different haplotypes invaded the park about the same time over the 19-year lag phase. The overall number of patches started to grow rapidly for all haplotypes at the similar time with different increasing rate, which resulted in different overall number of privet with different haplotypes. A saturation phase, likely caused by occupation of most suitable habitat, was similar for all haplotypes. Although there was a big difference in the number of privet carrying different haplotypes, there is no sign that one subgroup of privet was inhibited by another, according to the population growth curve (Fig. 14).

We generated distribution maps of haplotypes of 316 privet samples in the park at all the cpDNA markers. The maps did not suggest a significant spatial separation of *L. vulgare*

plants with different haplotypes. After overlaying the haplotype distribution map and cover-type layer, we did not find a spatial coincidence of haplotype distribution with the cover-types during the whole invasion history. Three samples carrying minor haplotypes, H4 and H6, were located in the northwestern of WMP and on sample carrying H5 was found in the north central of the park (Fig. 15).

To further understand if there is an association between haplotypes and the spatial distribution patterns, we performed spatial analysis by each haplotypes from all the markers. In a previous study, we described the dynamics and temporal-spatial pattern during the invasion process for all the privet individuals we recorded (Chapter 2). We compared the spatial patterns by different haplotypes and compared with the spatial patterns we discovered with all the privet individuals from previous study (Table 6). After performing nearest neighbor analysis, which tells how the points in the studied area spatially distributed, we found that the spatial distribution patterns are different among the samples carrying different haplotypes at the same marker and also different from the spatial pattern by all the samples (Table 6). The privet samples having haplotype 1 at trnS and haplotype 1 at combined marker were clustered within the same area. The privet samples carrying haplotype 3 at trnS, haplotype 2 at trnH and haplotype 3 with combined marker were geographically dispersed within the same area in WMP. The samples with the other haplotypes all randomly distributed over the landscape of the selected area. Our previous study showed that all the privet samples we collected randomly distributed over the selected area within the park after at least 39 years of invasion and there was no

record of clustering during the whole invasion history (Table 6).

Spatial autocorrelation based on the sample locations and their age information was analyzed by Moran's I to evaluate the spatial distribution pattern of age with respect to whether older and younger privet patches tend to be more spatially closed to each other. Otherwise whether privet patches with similar ages indicate tendency to be more far away from each other than expected distance. Different age spatial patterns were found among different haplotypes at all the markers. With respect to trnH, the samples carrying H 1 showed a significant clustered pattern of age spatial distribution. For trnS and combined marker, H 1 indicated a significant clustered age spatial pattern while H2 and H3 showed random spatial pattern. In addition, significant spatial autocorrelation was indicated by overall samples within the selected area with even higher Z-score.

Discussion

cpDNA diversity and haplotype distribution across states

We revealed considerable level of variations, which provided acceptable amount of resolution, using the three cpDNA markers to analyze the haplotype diversity of *L. vulgare* and some other *Lugstrum* species or cultivars from nursery. We demonstrated geographical structure over the region of three states with these cpDNA markers. However, we found no significant genetic divergence among *L. vulgare* sampled at six

sites in Ohio. Many years of continuous introductions and lack of geographical barriers may have facilitated the widespread distribution of all cpDNA haplotypes within Ohio. Limitation of long distance dispersal of seeds may inhibit the gene flow between the Ohio population and populations in eastern two states.

We observed a large-scale haplotype spatial distribution pattern that reflected the genetic consequence of invasion history. The decrease of genetic diversity of the eastern two states compared to Ohio sites possibly resulted from founder events during invasion across states. According to Cothran (2003), *L. vulgare* was probably introduced and first naturalized in eastern states. The haplotype geographical distribution pattern is consistent with the historic record, suggesting the expansion pathway of *L. vulgare* from east to west. There can be another explanation to the geographical genetic pattern. Multiple introductions of new genetic variants could also possibly have increased the haplotype diversity in New York and Pennsylvania populations. According to the haplotype network, H3 at trnH and H9 at combined marker that were highly genetic different probably came from separate introductions. Later introduction of those haplotypes at these sites may have resulted in the limited geographical range compared to the dominant haplotypes in Ohio. In this scenario we expected to see lower frequencies of newly introduced haplotypes due to adaptation and competition. However, in the two eastern states the suspected new haplotypes had similar or even higher frequencies than the dominant haplotypes within populations. Further sampling in more local populations in New York and Pennsylvania and sampling in more states may be helpful in providing

stronger evidences of the invasion history with respect that *L. vulgare* spread from eastern coast to west.

There were more haplotypes identified at the RR site in Northern Ohio than at KKL in New York. This may have resulted from the lower number of KKL samples and their limited geographic scale that did not cover significant genetic variation in New York. In addition, popular home gardening near site RR may have provided more diverse genetic sources, whereas the KKL site is mostly wooded and non-residential.

Age and genetic analysis in Wooster Memorial Park

The mixed pattern of haplotype distribution in WMP may have resulted from strong source populations and the influences of multiple satellite colonies. This speculation is consistent with our study over the broader state scale, suggesting a thorough distribution of all the haplotypes across Ohio and similar genetic configuration as most of the regions in Ohio. Our previous study also showed that privet patches formed a dispersed spatial pattern and were found across different positions of the park during the early period of invasion. Multiple immigration events of privet from one or more source populations into the park may have occurred during invasion, as opposed to an initial invasion and subsequent spread throughout the park from that site. Due to the comparatively slower rates of mutation, cpDNA markers are better used for characterizing genetic variations over large spatial scale. Therefore, fine spatial scale of genetic data may exhibit high

noise using cpDNA marker (Anderson *et al*. 2010). Future investigation of spatial genetic structure in WMP could be made using nuclear markers with higher resolution, which can also provide different perspective as the nuclear genome is dispersed by both pollen and seed.

Our results of age distributions and similar pattern of patch expansion for different haplotypes suggested that *L. vulgare* with different haplotypes invaded into WMP during the same period. The similar growth pattern of patch numbers among haplotypes, especially the coincident initiation of the period or rapid expansion, may be a result of a change in environment at that time. The increasing number of patches after lag phase was different among haplotypes generating different numbers of patches. This may suggest variation in fitness of *L. vulgare* associated with different haplotypes. The oldest patch in the park appeared in 1972; the next patch identified with that same haplotype occurred at least 16 to 17 years later. According to the record of site characteristics, the oldest plant was located in a maple forest at the edge of the park adjacent to farmland. Only 4 other privet patches were found near it, based on the maps with sample points (Figure 4 in Chapter 2) and only one of them carried the same haplotype (Figure 15). It is likely that certain landscape features that we cannot identify inhibited the spread from the oldest plant.

The different spatial pattern of different haplotypes, resulted from nearest neighbor analysis, may suggest an evidence of underlying spatial processing associated with the

different haplotype diversity we discovered. With respect to control of invasive plants, a dispersed spatial pattern may cause more effort while a clustered pattern is more easily discovered and eliminated. Therefore, we speculate that development of a control strategy can benefit from the understanding of spatial pattern of privet invasion that was associated with genetic diversity. The result from spatial autocorrelation by Moran's I analysis may indicate that the difference of spatial age distribution might not be associated with haplotype difference while environmental factors, such as disturbance and isolation, may impose greater effect (Chapter 2). The higher clustered age spatial pattern with overall samples at the same time lower clustered pattern and even random pattern with haplotypes may suggest that during the early invasion process, favorable habitats facilitated the colonization of privet. In other words, though the samples carrying specific haplotypes did not exhibit spatial autocorrelation with age or exhibit lower spatial autocorrelation, due to the same preference of habitats a higher clustered age spatial pattern was indicated after combining all the samples with different haplotypes. On the other hand, due to the inhibition of unfavorable habitats and extensive usage of favored habitats, new privet patches formed during the late invasion process.

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Site name	Site description	Number of samples	cpDNA analysis across states	Haplotypes coded in GIS	Age determination and spatial analysis
WMP	Wooster Memorial Park	345	24	316	331
GH	Glen Helen	24	24		
DC	Darby Creek	24	24		
HB	Highbanks Reservation	24	24		
RR	Rocky River	24	24		
LC	Lake County	23	23		
PT	Frick, Schenley, (Pittsburgh, PA)	24	24		
KKL	Keuka Lake, NY	14	14		

Table 2. Number and sites of samples and what analyses were performed with them. The 24 samples from WMP for cpDNA analysis across states were included in the overall 345 samples and were selected from different positions in the park. Missing samples for haplotype characterization were due to technique problems in PCR or sequencing.

cpDNA markers	Number of polymorphic sites	Number of haplotypes in study sites	Number of haplotypes in nursery	Total Number of haplotypes
trnS	16	6	7	11
trnH	22	5	7	10
Combined marker	38	11	7	16

Table 3. cpDNA markers and haplotype numbers.

Name	trnH	trnS	Combined
L. amurense cv. Amur River	H2	H3	H3
L. delavayanum	H6	H9	H16
L. ibolium (a)	-	H4	-
L. ibolium (b)	H2	H3	H3
L. japonicum	H7	H5	H12
L. lucidum cv. Davidson's Hardy	H8	H6	H13
L. quihoui	H9	H7	H14
L. x Vicaryi	H3	H4	H9
L. vulgare cv. Pendulum	H10	H8	H15

Table 4. Haplotypes of different privet species and cultivars. (a) and (b) indicate two unknown cultivars of *L. ibolium.* As nursery record, *L. ibolium* is a cross between *L. ovalifolium* and *L. obtusifolium*. *L. x Vicaryi* is a cross between *L. ovalifolium 'Aureum'* and *L. vulgare.*

Site name	N	S	Hd	K
WMP	4	5	0.7191	2.3143
GH	6	5	0.7524	2.0762
DC	3	5	0.4952	0.8762
HB	3	5	0.6810	2.2048
RR	6	36	0.7286	7.1286
LC	3	5	0.6277	2.0996
OH	9	36	0.6788	2.8197
PA	6	30	0.7708	12.8775
NY	4	28	0.6264	12.0110

Table 5. Genetic diversity of study sites and in three states. N indicates number of haplotypes; Hd indicates the haplotype diversity; S indicates number of polymorphic sites; and K represents the average number of nucleotide differences. In analysis, gap was considered as the 5th state.

Marker and Haplotype		Nearest Neighbor			Moran's I		
		Z score	P value	Pattern	Z score	P value	Pattern
trnS	H1	-1.9546	0.0504	Clustered	2.0138	0.0440	Clustered
	H2	-0.5978	0.5500	Random	0.6309	0.5281	Random
	H3	2.0797	0.0376	Dispersed	1.3487	0.1774	Random
trnH	H1	-1.3297	0.1836	Random	2.7448	0.0061	Clustered
	H2	1.7548	0.0793	Dispersed	1.0639	0.2874	Random
Combined	H1	-2.1265	0.0335	Clustered	1.7188	0.0856	Clustered
	H2	-0.7648	0.4444	Random	0.6116	0.5408	Random
	H3	2.1938	0.0282	Dispersed	1.2155	0.2242	Random
All	All	-0.8448	0.3982	Random	4.8088	0.0000	Clustered

Table 6. Spatial analyses in selected rectangular area with samples carrying different haplotypes at each marker and all the samples. Only H1, H2 and H3 for combined marker were included in the spatial analyses because of the low number of samples carrying H4 to H6.

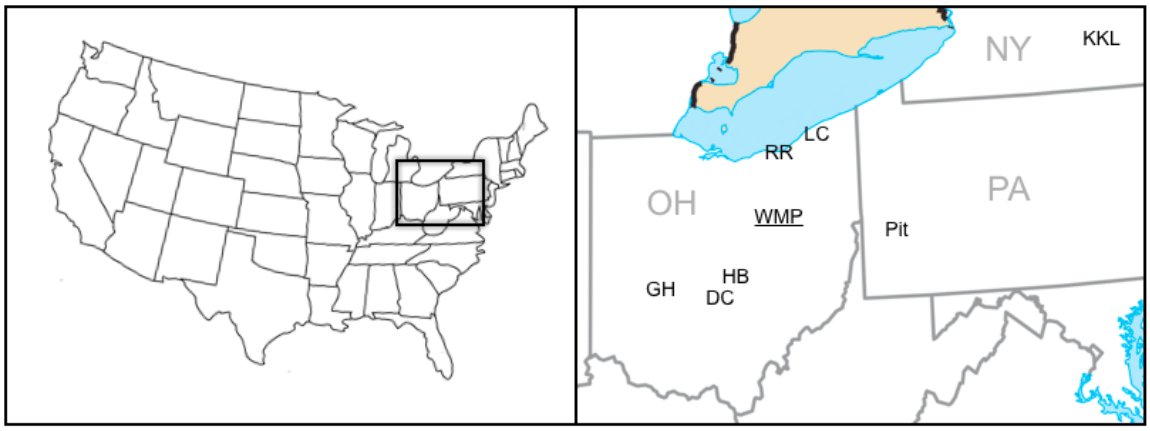

Figure 10. Geographic locations of all collection sites. The positions of abbreviations indicate the approximate geographical locations of collection sites. Wooster Memorial Park (WMP) is underlined in the map.

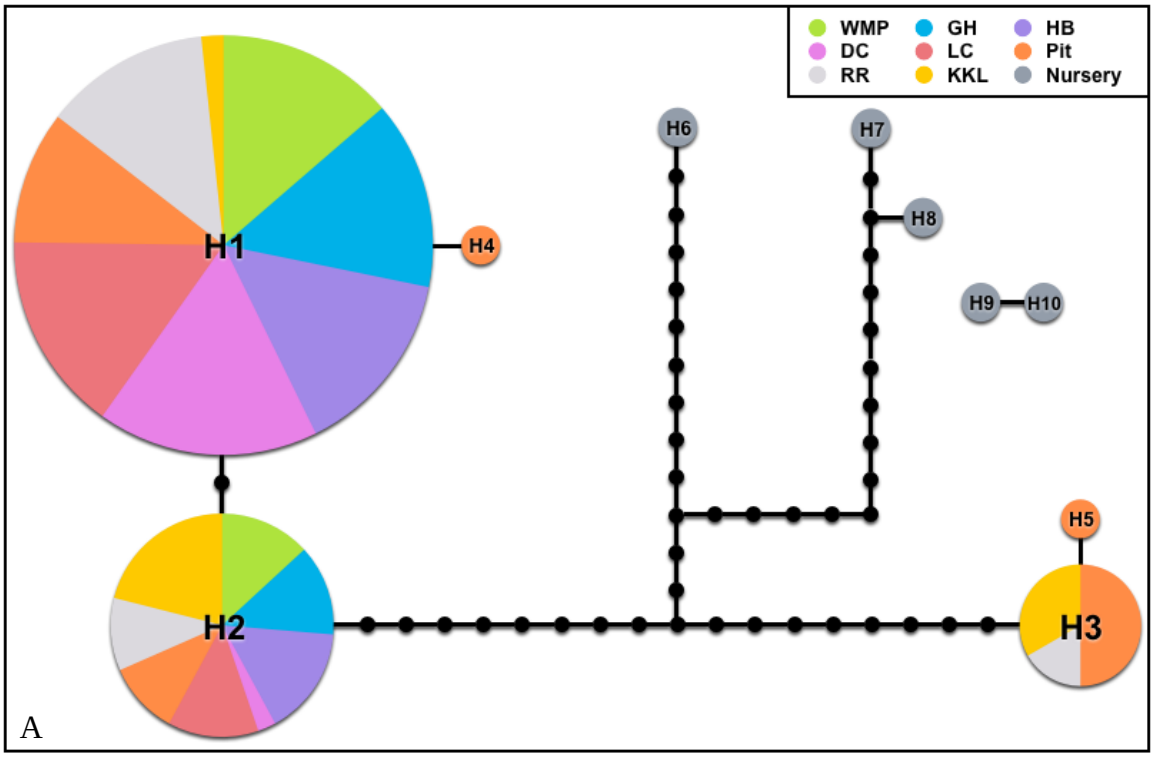

Figure 11. Haplotype networks at trnH, trnS and combined marker. Haplotype networks at trnH (A), trnS (B) and combined marker (C). Pie charts with different colors represent the populations sampled. A haplotype is represented by a circle, whose size is proportional to the number of individuals having that haplotype. Each line between points and circles indicates a mutation event. Haplotypes only found in nursery samples are in dark grey color. Haplotypes not connected by a dash can not be connected with 90% parsimony. Gaps were considered as the 5th state in haplotype network construction.

Continued

Figure 11 continued

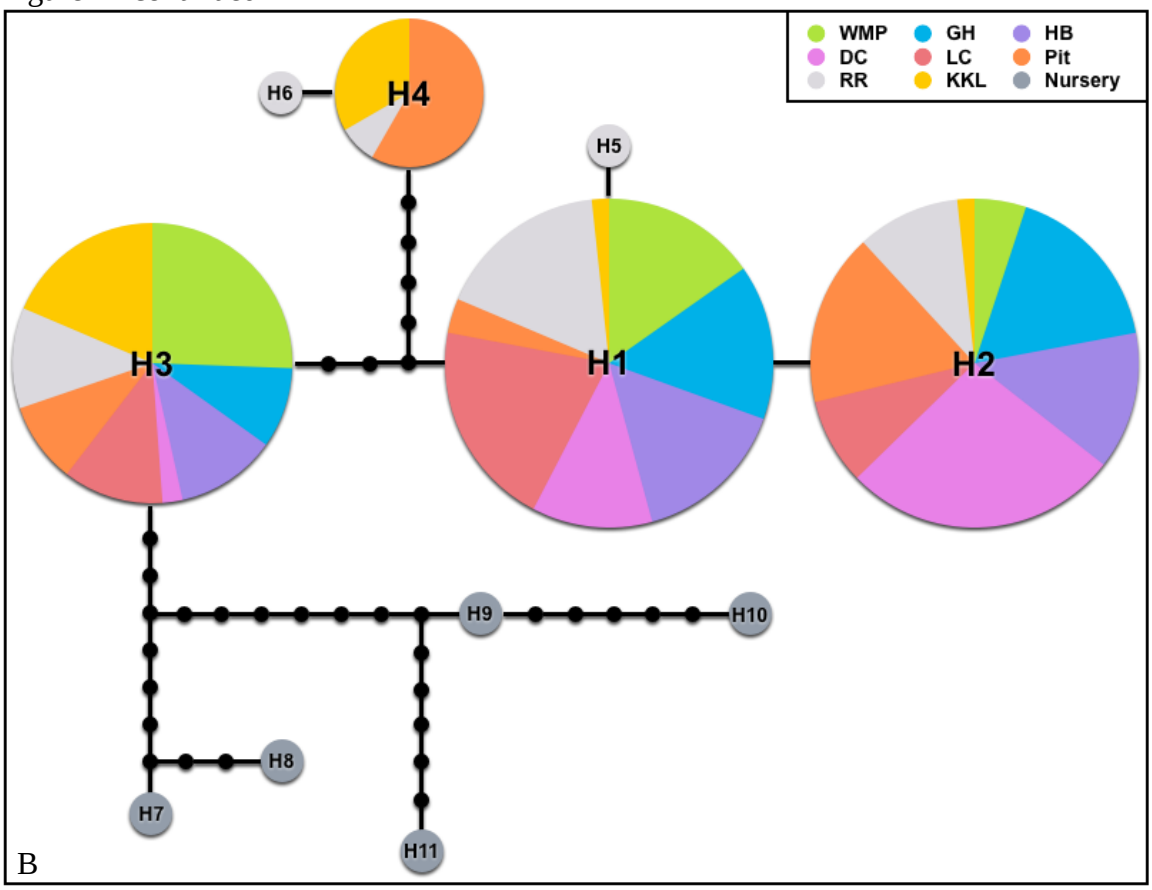

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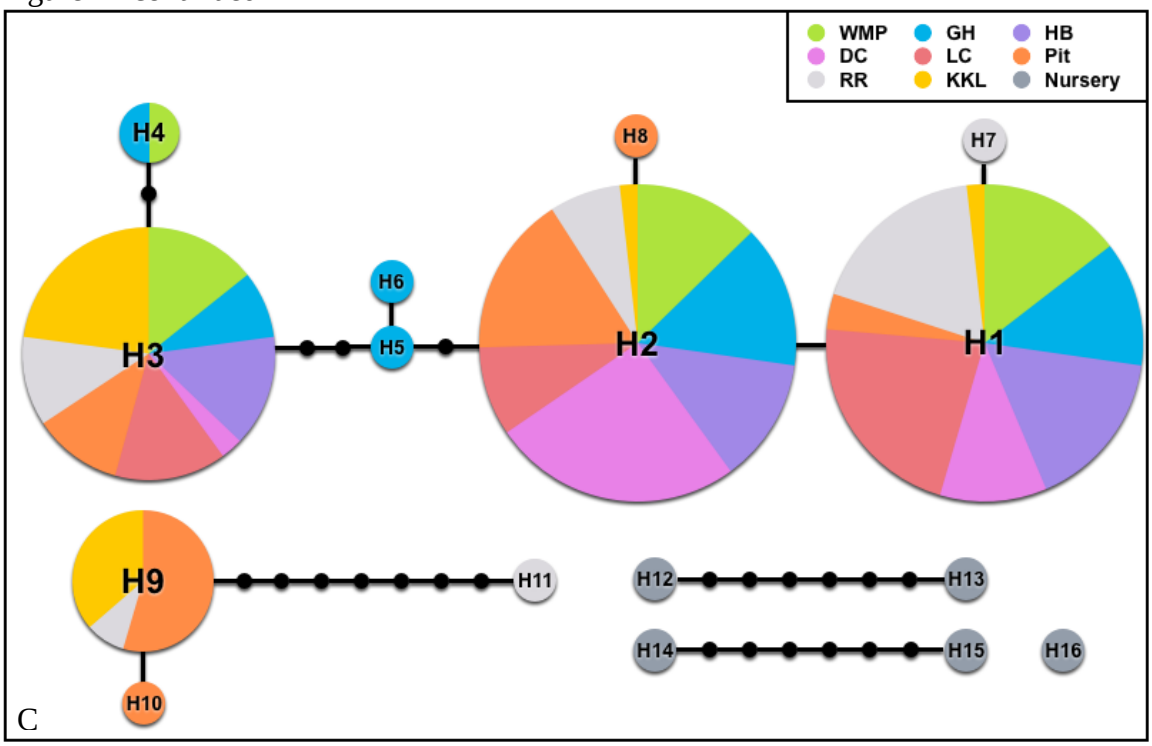
Figure 11 continued



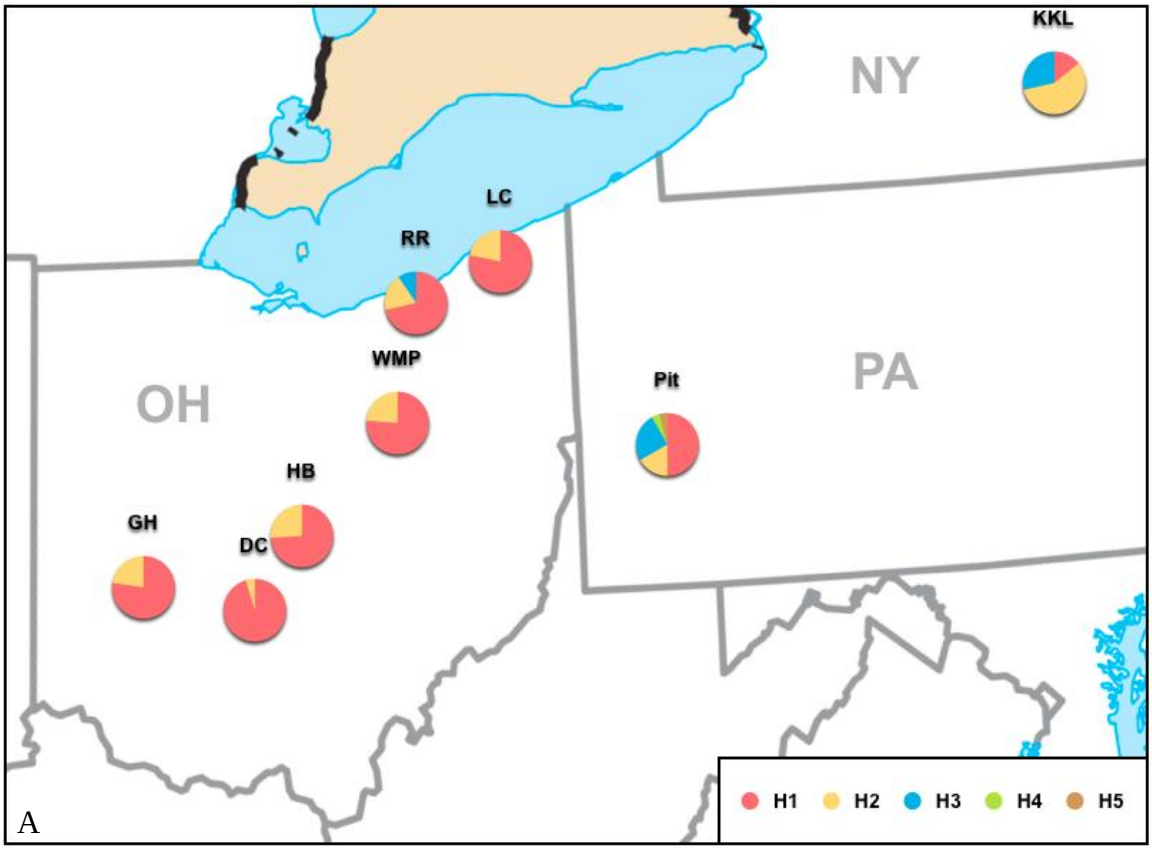


Figure 12. Distribution frequencies of cpDNA across three states. Distribution frequencies of cpDNA haplotypes at trnH (A), trnS (B) and combined marker (C) across three states. The approximate geographic locations of sampled populations are indicated by the positions of pie charts. Pie charts represent the frequency of haplotypes in each population.

Continued

Figure 12 continued

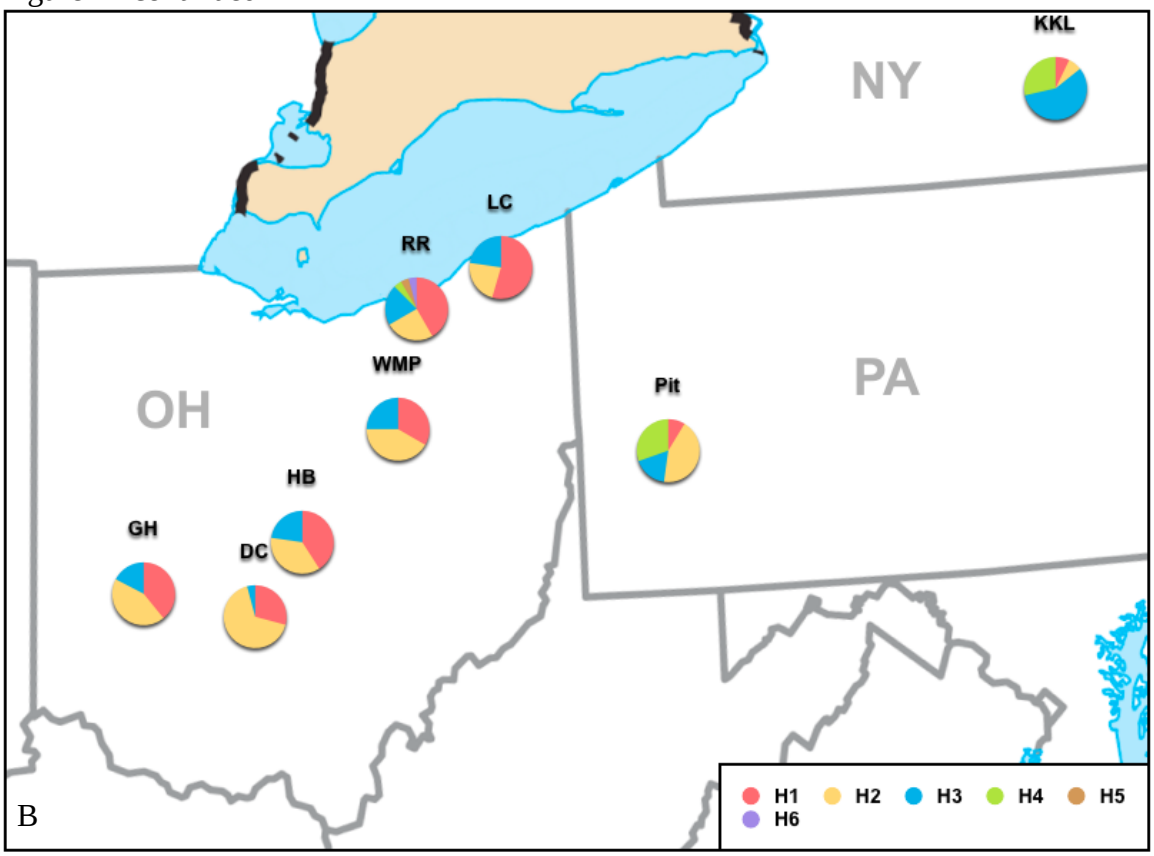


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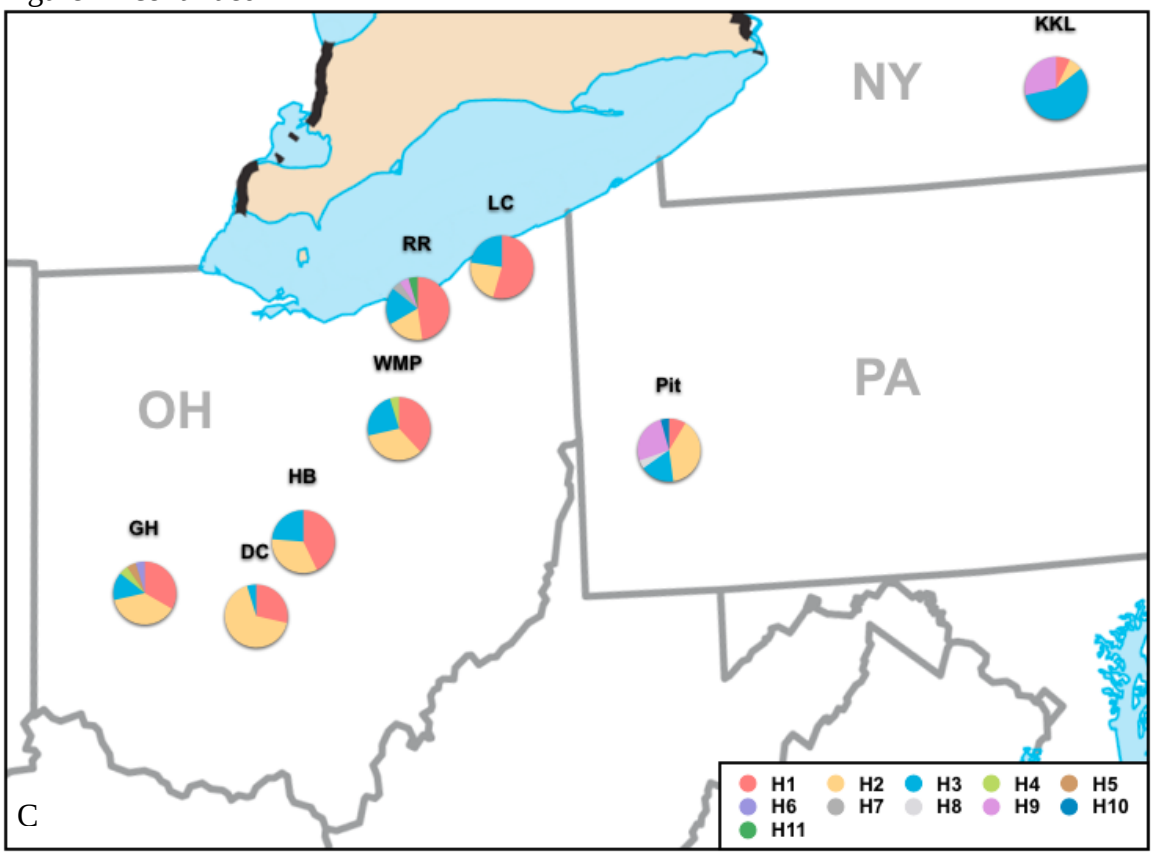
Figure 12 continued



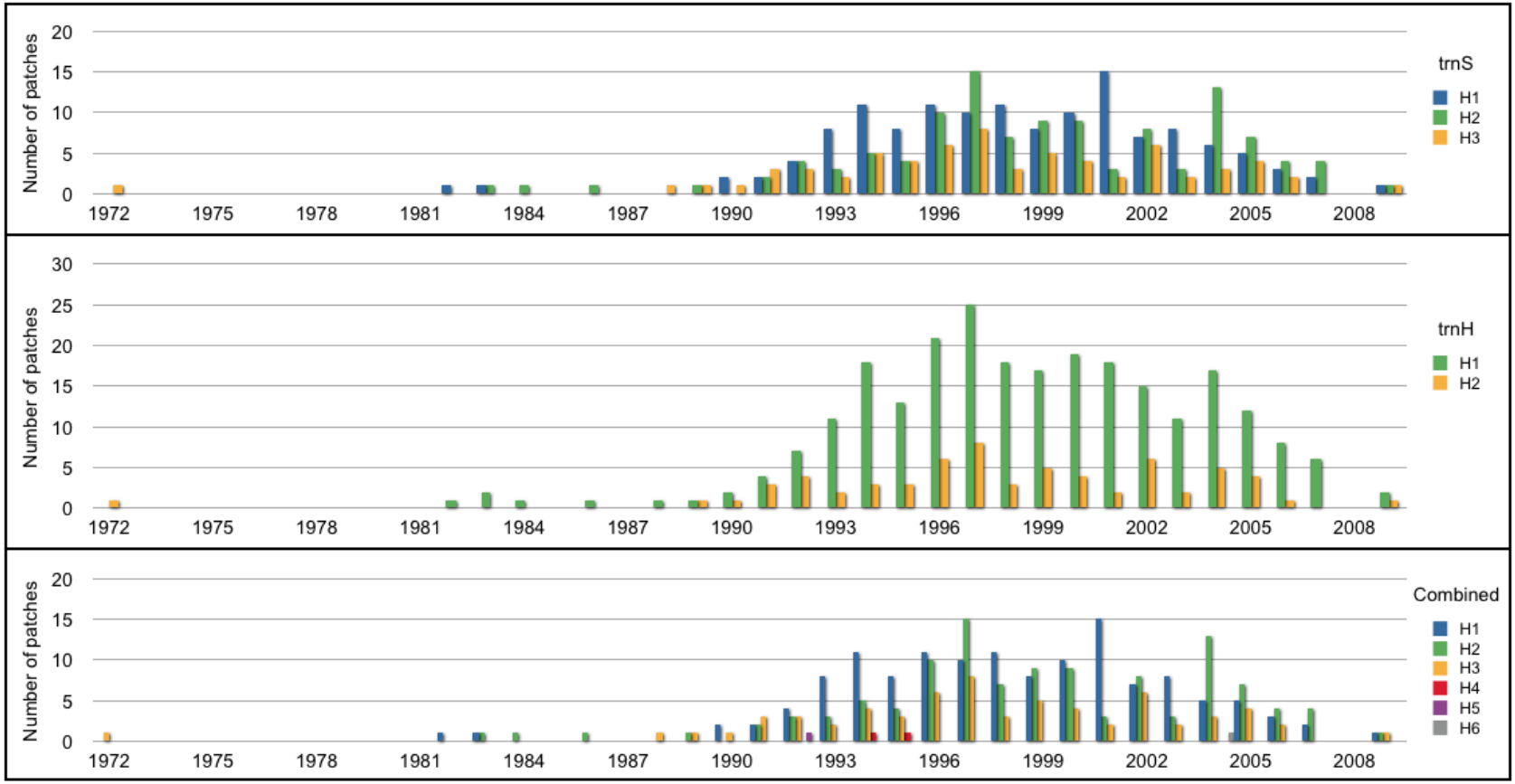


Figure 13. Age distribution by different haplotypes at three markers.

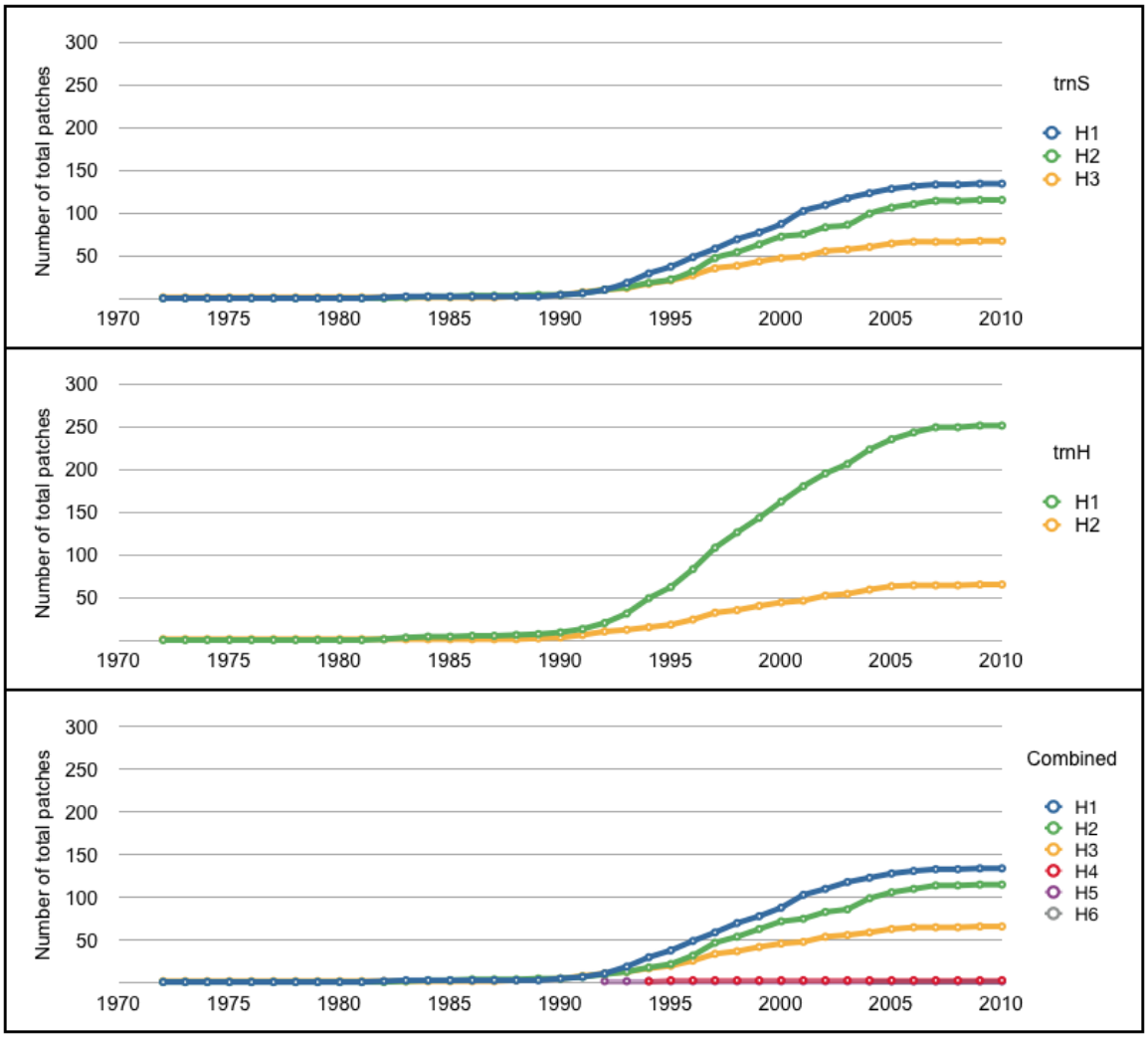


Figure 14. Invasion process of *L. vulgare* by different haplotypes.

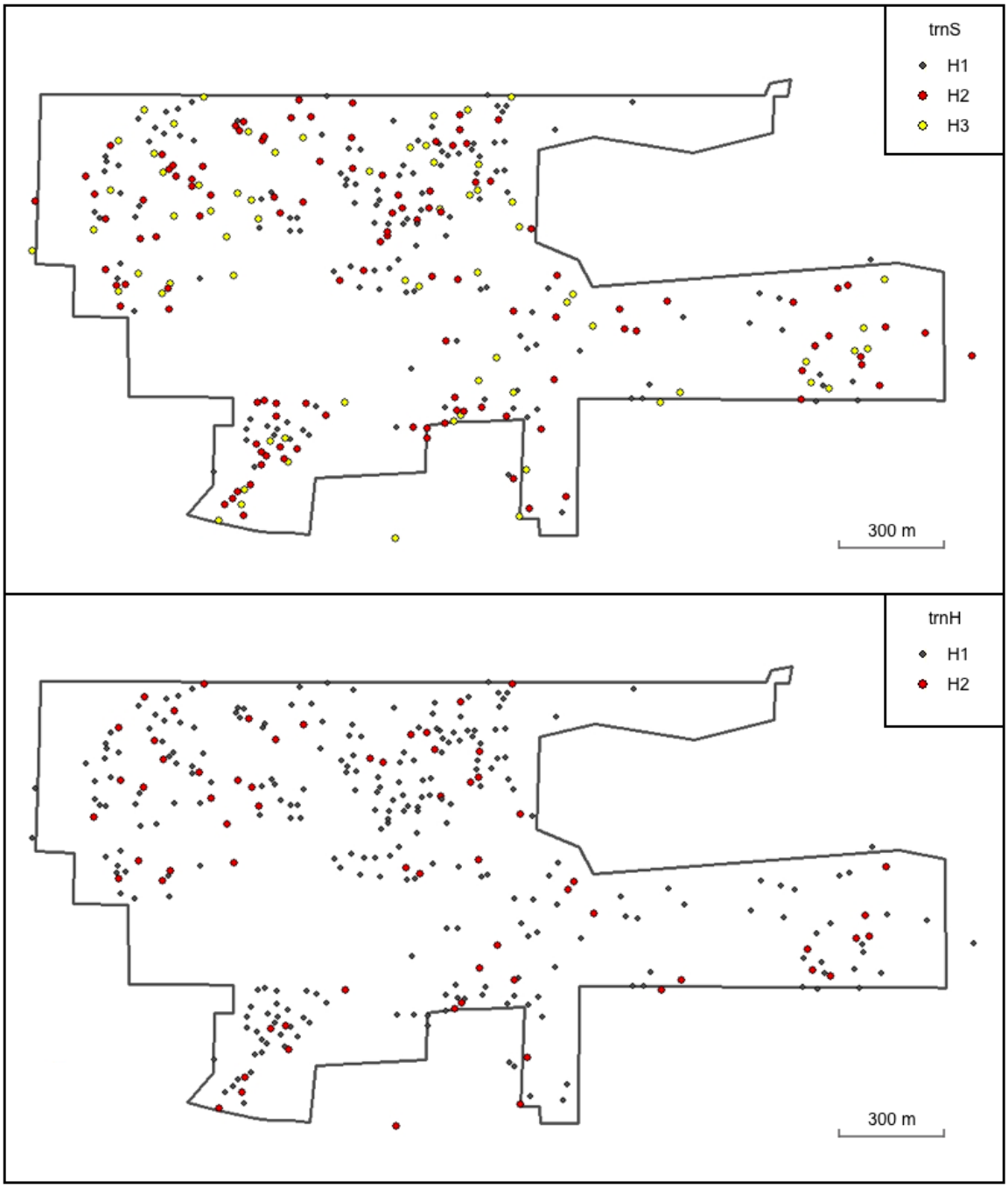
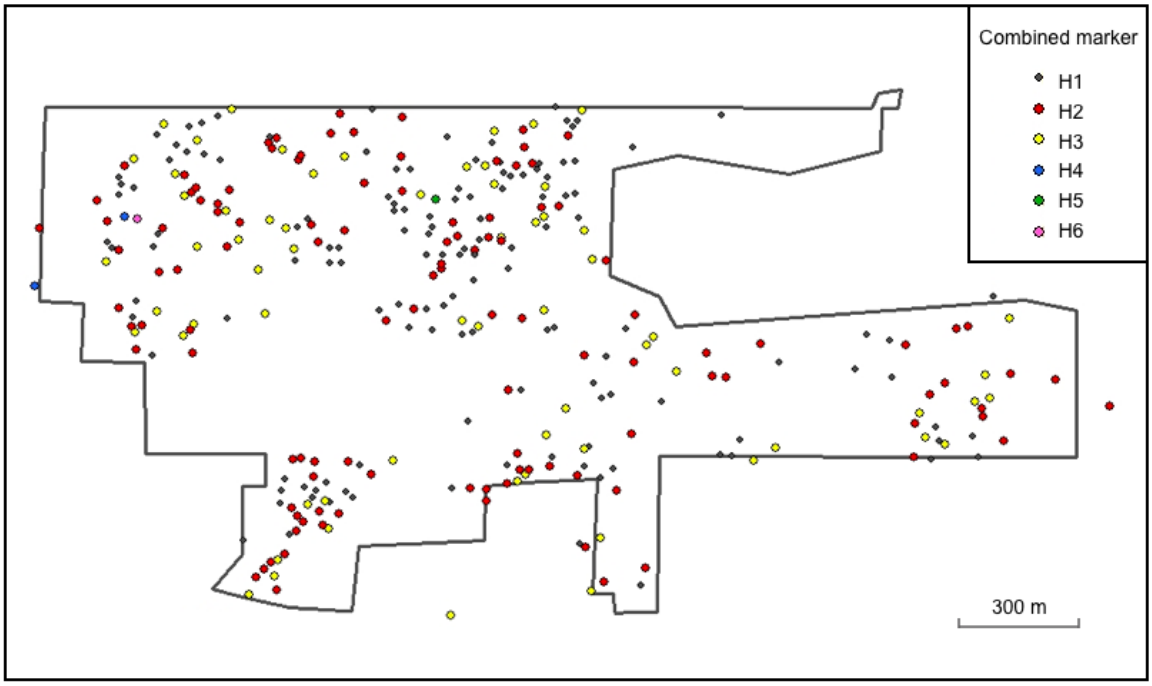


Figure 15. Haplotype distribution maps in Wooster Memorial Park at different markers.

Continued

Figure 15 continued



Chapter 4: Summary, Conclusions and Recommendations

Due to the complex of landscape features and lack of understanding of spread behavior of invasive plants, land managers need overall control strategies instead of the simple search-and-kill methods. Information on the genetic, spatial, and temporal patterns of plant invasion is important for development of invasive management, yet efforts to link the three factors to obtain comprehensive understanding of invasion history is lacking. In this study, we investigated the genetic structure, age distribution, and spatial pattern of an invasive plant, *Ligustrum vulgare* (common privet), and reconstructed the invasion history on different geographic scales by linking these factors.

We conducted a comprehensive survey in our main study site, Wooster Memorial Park (WMP), locating and mapping all the patches by GPS. Core stems samples for age determination and leaf tissues for DNA extraction were collected from the biggest plant in each patch to represent the putative oldest plant in that patch. We also collected and extracted DNA from14 to 24 leaf samples from eight sites in Ohio, New York, and Pennsylvania. In WMP we successfully determined the age of 331 samples and extracted DNA from 316. We characterized three chloroplast DNA markers from a previous study and used them to identify haplotypes from all the samples in WMP and in other study sites and samples from a commercial nursery. We demonstrated the geographic

distribution of haplotypes over three states and how samples with different haplotypes spread over landscape of WMP. Spatial analyses to estimate clustering status were performed with all the samples in partial area in WMP and with groups of samples carrying different haplotypes. Average age and growth rate in different cover-types were compared to investigate effect of habitats on invasion process.

We successfully reconstructed invasion history of *L. vulgare* within WMP. Based on the increasing rate of patch number, we discovered three phases of 39-year invasion process with respect to a 19-year long lag phase, a 16-year rapid growing phase, and a short phase suggesting saturation of habitats. We found that the favorable habitats for the initial patches established during the lag phase were located at edge habitats and opening areas. The privet patches were randomly distributed over the park, and there was no clustering, suggesting the lack of an intense invasion in a specific spot or an invasion front. The random spatial pattern may result in the fast invasion over the entire park area. The spatial distribution in the rectangular area showed a similar pattern. Although approaching a clustered distribution pattern, mostly it presented a random spatial pattern. However, clusters of old patches and young patches were found in the rectangular area, suggesting the possible corridor and barrier of the invasion. The uneven spatial distribution by age was not significantly associated with cover-type, though the cover-type providing different growing conditions produced different growth rates.

Different spatial distribution patterns were associated with the different haplotype; however, the spatial clustering pattern of age was not associated with haplotype. Samples with different haplotypes colonized and naturalized in WMP during the same period. A change in environment may have resulted in the coincident initiation of the rapid expansion of all the haplotypes. WMP may be under a great invading pressure from a strong source population, which resulted in the randomly mixed pattern of haplotype distribution and multiple immigrations.

Results revealed considerable genetic variation among *L. vulgare* and other *Lugstrum* species or cultivars from nursery in this study. We found a decrease in haplotype diversity from eastern two states to Ohio sites, which possibly resulted from founder events during invasion across states. The haplotype geographical distribution pattern is consistent with the historic record, suggesting the expansion pathway of *L. vulgare* from east to west.

Our findings in this study provide practical information for invasive management. The long lag phase of woody perennial invasive species provides a best time for controlling, however the small number of plants in the lag phase make them difficult to detect. The discovery of favorable habitats for initial patches during the lag phase can assist early detection of *L. vulgare* or even other invasive plants. In addition, the exponential growth rate of patches is important information for predicting the timing and level of control effort. The initial patches that were highly dispersed over the park may contribute to the

spatial pattern at the late stage of the invasion. Therefore, mapping spatial distribution patterns of invasive plants in early stages may help predicting spatial distribution pattern in later stages. We found that spatial distribution pattern was not associated with cover-types. However, the mapping of clusters of old and young patches may assist in invasive control effort in identifying invasion hot spots and cold spots.

We recognize that there are several limitations to the studies described in this thesis. Our sampling method in patch identification may not be accurate. Younger patch were difficult to distinguish when they might have been connected with or overlapping a larger patch. The size of the park area may affect the estimation of lag phase and age spatial distribution. We were not able to perform spatial analyses over the entire park area due to the shape the park. Due to the comparatively slower rates of mutation, the cpDNA markers we used in this study may not present enough polymorphism within the park.

Some additional research is needed. Further sampling in more sites in New York and Pennsylvania and sampling in more states may be helpful in providing stronger evidence of the invasion pathway. Nuclear makers showing more polymorphisms can be used to obtain higher resolution within WMP. Analysis of nuclear makers compared with cpDNA markers may provide different perspective as the nuclear genome is dispersed by both pollen and seed. Study in other parks with larger or different landscape features could reveal more information about interactions between age, spatial pattern, and landscape features.

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