Landscape influences on dispersal of white-tailed deer and attendant risk of chronic wasting disease spread as assessed by a landscape genetics approach

by

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CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

This thesis consists of four chapters, two of which are manuscripts to be submitted to peer-reviewed journals. In Chapter 1, I provide a general introduction to my research, and Chapter 4 contains the general conclusions of my research and implications for management. Chapter 2 focuses on the population genetic structure of white-tailed deer in northeastern Iowa and southwestern Wisconsin, the effect of the Mississippi River on genetic connectivity between these two states, and interpreting implications with respect to risk of chronic wasting disease spread from Wisconsin to Iowa. Chapter 3 examines the effect of an agriculturally-dominated landscape on genetic structure of female white-tailed deer. Chapters 2 and 3 are intended to be modified slightly and submitted for publication. This thesis was the product of my own personal work of data production, analysis, and writing. My major professor, Dr. Julie Blanchong, is listed as a co-author on the manuscripts (Chapters 2 and 3) because she provided guidance and expertise for this project.

General Introduction

Understanding factors that influence the spread of wildlife diseases is crucial for designing effective surveillance programs and appropriate management strategies. The potential introduction of chronic wasting disease (CWD), a fatal neurodegenerative disease of cervids, to Iowa is of significant management concern because it is found in several

bordering states including Wisconsin, where it was first detected in free-ranging white-tailed deer (*Odocoileus virginianus*) harvested in 2001 (Joly et al. 2003). CWD has subsequently been detected in Grant County WI, which borders Iowa, with one positive case in both 2006 and 2008 (WDNR 2010).

I studied the effect of the Mississippi River, which separates Wisconsin and Iowa, on deer population genetic structure in the two states (Chapter 2). My main objective was to characterize the degree of genetic connectivity between deer populations in Iowa and Wisconsin to identify factors influencing the risk of CWD entering Iowa through the natural movement of free-ranging deer from Wisconsin. I hypothesized that the Mississippi River would restrict deer gene flow between the states and thus spread of CWD by dispersing, infected individuals.

To better understand the potential for CWD spread in Iowa, if it were to be detected in the state, I studied the effect of the agricultural landscape in northeastern Iowa on deer population genetic structure (Chapter 3). White-tailed deer dispersal, and therefore population connectivity and degree of genetic structure, is likely influenced by the presence and configuration of their preferred forest habitat. My main objective was to characterize the population genetic structure of white-tailed deer in northeastern Iowa and understand how an agriculturally-dominated landscape may be affecting that genetic structure. I hypothesized that deer spatial genetic structure in Iowa, where 67% of the land is agricultural and only 9% is forested, is weaker than genetic structure of deer in more forested landscapes.

I used a landscape genetic approach to address my study objectives. Landscape genetics is the identification of correlations between population genetic structure and landscape features (Manel et al. 2003). It is a valuable way of assessing landscape effects on

wildlife population genetic structure and patterns of connectivity that may influence the spread of diseases. I used female deer in this study because they are traditionally considered to be the philopatric sex (Hawkins and Klimstra 1970, Hirth 1977, Porter et al. 1991, Purdue et al. 2000) and are expected to show a stronger signal of local genetic structure than males, therefore maximizing my ability to detect population genetic structure.

The total study area consisted of three southwestern Wisconsin counties along the Mississippi River and fifteen northeastern Iowa counties adjacent to and inland from the river (Fig. 1). The study areas for the two different portions of my study are slightly different, but

Figure 1. Total study area for my project in southwestern Wisconsin and northeastern Iowa.

overlapping. For the first objective (Chapter 2), addressing the effect of the Mississippi River on deer gene flow, the study area consisted of the three Wisconsin counties and four Iowa

counties across the river from them (Allamakee, Clayton, Delaware, and Dubuque). For the second objective (Chapter 3), understanding the effect of an agricultural landscape on deer spatial population genetic structure in Iowa, the study area consisted of all fifteen of the Iowa counties. Specific details of each of these study areas are presented in subsequent chapters.

Chronic Wasting Disease (CWD)

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) caused by a proteinaceous infectious agent referred to as a prion. It is related to other TSEs that affect ruminants, such as bovine spongiform encephalopathy (BSE, “mad cow disease”) and scrapie of sheep and goats, that are of significant economic concern, and in the case of BSE, of considerable public health concern as well (Williams et al. 2002). Several cervid species are known to be affected by CWD including white-tailed deer, mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) (Miller et al. 2000), and moose (*Alces alces*) (Kreeger et al. 2006). Because CWD is always fatal (Sigurdson 2008), it could have serious impacts on the viability of deer populations, and some modeling has suggested that CWD epidemics could drive deer populations to extinction in the locality of the epidemic (Miller et al. 2000). The maintenance of viable deer populations is of great concern to state wildlife agencies because deer hunting provides a major source of revenue in the form of deer hunting license sales for agencies such as the Iowa Department of Natural Resources, and also stimulates the local economy through other purchases by hunters (Stone 2003). There have also been concerns that, like its relative BSE, CWD could cross the species barrier and infect humans. Despite some human deaths that were originally suspected

to be related to consumption of venison from CWD-infected areas, no known cases of CWD affecting humans have been identified (Belay et al. 2004). However, concerns still remain in the minds of hunters about CWD-infected deer, and right after CWD was detected in Wisconsin in 2002 sales of deer hunting licenses in Iowa dropped (Stone 2003).

CWD is caused by a prion that is a misfolded naturally-occurring cellular protein known as PrP that facilitates the re-folding of other correctly folded PrP proteins into the misfolded configuration, and the accumulation of these misfolded (prion) PrP proteins in central nervous system tissue leads to neurodegeneration and ultimately death (Sigurdson 2008). Lymphoid tissues (especially retropharyngeal lymph nodes) and the obex (brain stem) are early sites of CWD prion accumulation in mule deer, after which prions accumulate in other parts of the central and peripheral nervous system and eventually in other tissues and organs (Fox et al. 2006).

The infectious CWD prions can be transmitted to other deer via saliva and blood (Mathiason et al. 2006), and laboratory studies have found that prions can also be shed in feces (Safar et al. 2008). Transmission of CWD from an infected individual to a susceptible one may require repeated exposures (Williams et al. 2002). It appears that the vast majority of transmission is lateral (Miller et al. 2000, Sigurdson 2008), with only about 3.4% vertical transmission between mother and fawn in mule deer (Miller et al. 2000). In white-tailed deer in the Midwest, males have a higher probability of becoming infected than do females, and disease prevalence increases with age, more so for males than females (Grear et al. 2006).

The potential for indirect infection via environmental transmission of CWD has also been studied. Uninfected deer can become infected with CWD after exposure to feed buckets, water, and bedding from the pens of CWD-infected deer, indicating that infection

solely by environmental transmission is possible (Mathiason et al. 2009). Additionally, uninfected deer have become infected with CWD when housed in paddocks where CWD-infected deer had previously resided 2.2 years earlier or in paddocks where carcasses of CWD-infected deer had decomposed 1.8 years earlier (Miller et al. 2004). Prions in the environment can exhibit extreme resistance to degradation, potentially remaining in the soil for years (Russo et al. 2009). However, persistence of prions in the environment has been connected to soil type, with soils high in manganese oxide resulting in oxidation and degradation of prions (Russo et al. 2009), and porous soils with more basic pH allowing greater movement of prions through the soil relative to movement in finer-textured, more acidic soils (Ma et al. 2007).

Once a deer becomes infected, the incubation period of the disease is estimated to be 18 to 24 months (Miller et al. 2000) followed by progressive onset of clinical signs(Williams et al. 2002). Earliest symptoms, such as changes in frequency of interactions with conspecifics, may be quite subtle and not apparent to unfamiliar observers (Williams et al. 2002). As the disease progresses, however, abnormal behaviors become increasingly obvious. These include repetitive movements, decreased food consumption (leading to a decrease in body condition from which the disease gets its name), increased salivation and drooling, increased drinking and urination, as well as changes in posture and coordination including drooping head and ears, wide-legged stance, stumbling, trembling, and general lack of coordination. The interval from onset of symptoms to death can be anywhere from just a few days to as much as a year, although a few weeks to 3-4 months is most common (Williams et al. 2002).

Mule deer displaying symptoms of the disease that has come to be known as CWD

were first recognized in captive herds at research facilities in Colorado during the late 1960s (Williams et al. 2002). The first cases of CWD in free-ranging cervids were identified in free-ranging elk in 1981, mule deer in 1985, and white-tailed deer in 1990 in northeastern Colorado and southeastern Wyoming (Williams et al. 2002). The disease has spread geographically over the past four decades and has currently been identified in free-ranging populations of cervids in eleven U.S. states (Fig. 2) and two Canadian provinces (CDC 2010). The current distribution of the disease appears to be partly related to natural movements of free-ranging deer and elk and partly a result of commercial movement of infected captive animals from one geographic region to another (Belay et al. 2004) as a result of inadequate regulations (Williams et al. 2002). Indeed, it is considered likely that the movement of an infected captive cervid into Wisconsin was the initial cause of the detection of the disease east of the Mississippi River (Joly et al. 2003).

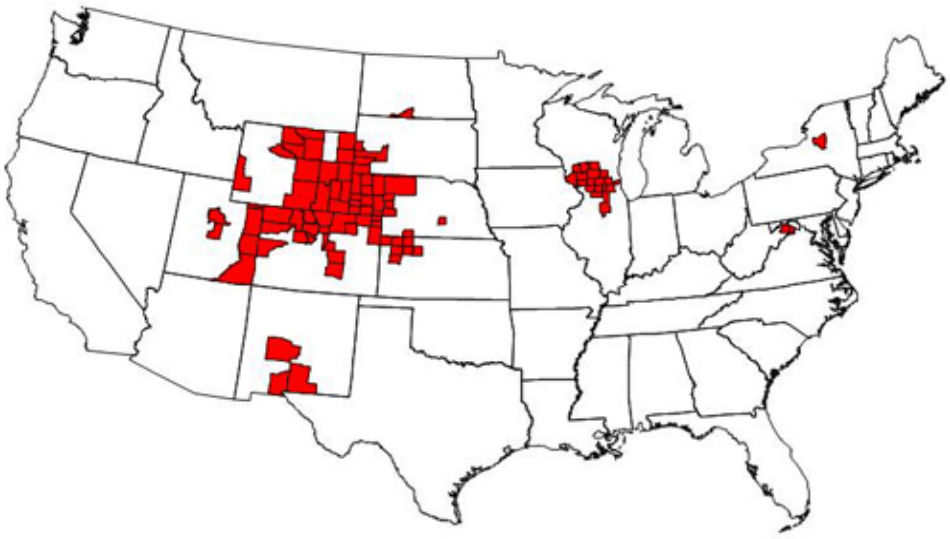

Figure 2. Counties where CWD has been detected in free-ranging cervids in the United States as of March 2010 (CDC 2010).

Since the discovery of CWD in free-ranging white-tailed deer in Wisconsin in 2001, in the Midwest it has also been found in captive Wisconsin cervids, free-ranging Illinois white-tailed deer, and captive elk in Minnesota (Joly et al. 2003). As of the fall of 2009, Iowa has not yet had any reported cases of CWD-infected cervids, based on surveillance from 2006 to the present, but transmission of CWD into the state by dispersing deer from neighboring infected states is of imminent concern to the Iowa Department of Natural Resources (IDNR).

The IDNR currently samples hunter-harvested deer for CWD all across Iowa and especially in the northeastern portion of the state closest to Wisconsin (Fig. 3; IDNR 2010). A few additional samples from roadkilled deer and targeted individuals displaying symptoms consistent with CWD are also tested (W. Suchy, IDNR, pers. comm.). Sampling schemes such as that conducted by the IDNR are extremely important because CWD prevalence can exceed 1% before clinical cases are first detected in an area (Miller et al. 2000). While the IDNR's current sampling regime may be sufficient to detect CWD before it reaches a prevalence much greater than 1% (if it enters the state at all), it is not necessarily the most efficient or effective method of focusing sampling resources. The IDNR wishes to fine-tune its sampling scheme to have the greatest possible chance of detecting CWD-infected deer at the lowest possible level of prevalence, given current available resources of money, time, and personnel. However, very little is known about dispersal of white-tailed deer, and attendant risk of CWD-spread, between Wisconsin and Iowa across the Mississippi River. Information is also lacking with regard to population genetic structure within northeastern Iowa. There may be a single population with regular gene flow throughout or multiple separate

populations with limited gene flow among them. Distance, direction, and rates of deer dispersal (gene flow) within northeastern Iowa could influence how rapidly CWD might spread across Iowa if it enters the state.

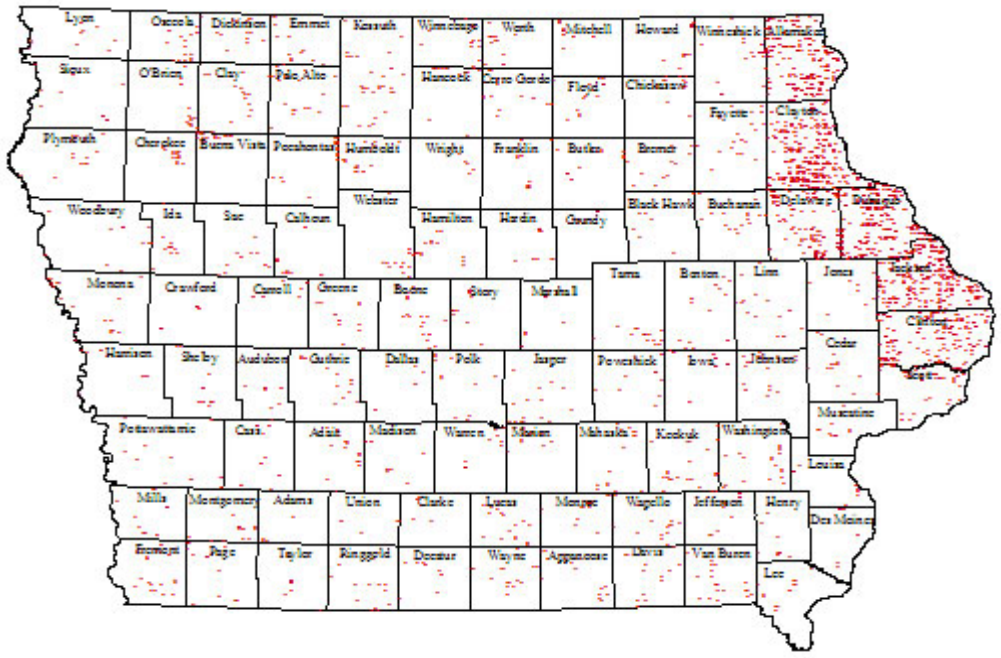

Figure 3. White-tailed deer samples collected in Iowa during the 2006-07 hunting season and tested for CWD (IDNR 2010).

Ecology of White-Tailed Deer

Habitat:

White-tailed deer are distributed across much of North America and occupy a wide variety of habitats, from deciduous and coniferous forests to more open ranges with broad plains or savannas (Hirth 1977). Forests and forest edges provide important cover for deer (Halls 1984) and in the more open ranges, brushy draws are important for cover (Hirth 1977). During the winter, especially if it is a harsh winter, deer rely primarily on forests for cover,

although brush, tall weeds, standing corn, and cattails can provide sufficient cover during the rest of the year (Halls 1984). Conifers can be important for winter thermal cover, and areas of early successional deciduous and mixed forests provide high potential for spring and summer habitat (Felix et al. 2004). Modeling approaches have shown that deer populations are sensitive to habitat components such as snow depth, patch area of forests that provide protective cover, and availability of forest edge habitat (Shi et al. 2006). Deer in an agricultural region of Illinois select early successional forest habitats and mixed forest cover that included both hardwoods and conifers during the winter and avoid bottomlands during all parts of the year (Nixon et al. 1991). Deer may avoid using bottomland forests during the summer because of the higher density of biting insects in this habitat compared to other habitats such as corn fields and forest edges (Nixon et al. 1991). Intensive farming in the Midwest and the trend toward fewer and larger farms with larger field sizes limits the amount and diversity of forested habitat available to deer, except along riparian systems and in more hilly areas (Halls 1984).

White-tailed deer in the Midwest have generally benefited from the abundant source of food provided by the intensive farming in the region, with crops such as corn and soybeans comprising a major portion of their diet (Halls 1984). Deer usage of agricultural fields for foraging mainly focuses on waste grain left in fields after harvest, but they will feed on crop plants such as corn, soybeans, and alfalfa throughout the entire growing season (Halls 1984). In a study of deer in an agricultural setting, Nixon et al. (2007) found that female deer fed longer in soybeans than in corn or other forage crops, but not for more than about two hours, and periodically returned to woody cover because of fawn rearing (Nixon et al. 1991). Males, on the other hand, spent extended periods, as much as a full day or more,

feeding in crop fields (Nixon et al. 1991). High quality woody browse (maple and cedar) and mast-producing hardwoods (oak and beech) can be important autumn and winter food sources for deer (Felix et al. 2007).

Dispersal and exploratory movements:

In white-tailed deer, males are traditionally viewed as the dispersing sex and females are typically philopatric, establishing adult home ranges in their natal area (Hawkins and Klimstra 1970, Nelson and Mech 1984). Dispersal of deer has been explored by numerous telemetery studies throughout the United States. Yearling males have been found to disperse during two distinct periods, the first is during the fawning period in the spring and the second is during the fall rutting season (Rosenberry et al. 1999, Diefenbach et al. 2008, Skuldt et al. 2008). The exact percentage of males that disperse varies somewhat from region to region, but is generally at least half of all yearling males and in some cases is considerably more. In an agricultural landscape in Illinois, 51% of males dispersed (Nixon et al. 1991). Males in partially (51-61%) forested landscapes in Pennsylvania dispersed at a rate of 46-74% (Long et al. 2005). In northern Minnesota, 70% of males dispersed by the age of 2 years (Nelson and Mech 1984), whereas 80% of males in southern Illinois dispersed (Hawkins and Klimstra 1970), and in Texas 90% of males eventually dispersed, although 15% were over 2.5 years old before they dispersed (Webb et al. 2007). Dispersal rate in males has been found not to be correlated with percentage of forest cover, but both average and maximum dispersal distance in males were highly negatively correlated ($R^2 = 0.94$ and $R^2 = 0.86$, respectively) with percentage forest cover in a meta-analysis of nonmigratory deer populations in several states (Long et al. 2005). Average dispersal distance for males has been documented to be around 7-

8 km in forested landscapes (Nelson and Mech 1984, Long et al. 2005) and average 40.9 km in an agricultural area of Illinois (Nixon et al. 1991). Maximum dispersal distances in partially forested landscapes are around 31-41 km in Pennsylvania (Long et al. 2005) and up to 58 km in Maryland (Rosenberry et al. 1999).

The percentage of females that disperse varies dramatically between studies and appears to be strongly influenced by the amount of forested habitat. Skuldt et al. (2008) observed only 3% dispersal (1 out of 32) in female yearlings in a 54-60% forested landscape in southern Wisconsin. Of female yearlings on a southern Illinois wildlife refuge with 27% brushland and 29% forest, 13% dispersed (Hawkins and Klimstra 1970). In west-central Illinois, where the landscape is 20% forested, as many as 39% of females dispersed, and in heavily agricultural landscapes in east-central and northern Illinois with only 1.6-2.7% forest, as many as 45-49% of females dispersed (Nixon et al. 2007). Female dispersal distance also appears to be related to habitat type. Female dispersers traveled from 4.5 – 8.0 km in a South Carolina site with 97% forest (Comer et al. 2005) and in southern Illinois landscapes with 66% brushland or forest females averaged 6.8-km dispersals (Hawkins and Klimstra 1970). However, in agricultural regions of Illinois with extremely little forest cover, females have been observed to disperse an average of 37-41 km (Nixon et al. 2007).

In non-migratory deer populations, dispersal from the natal range to establish an adult home range is the primary form of long-distance movement (Webb et al. 2007), however young deer that have not established adult home ranges will also occasionally make long-distance exploratory or transient movements that may not be true dispersal events (Oyer et al. 2007). In a predominantly forested landscape in south-central Wisconsin, deer of both sexes and all age classes, including up to 31% of yearling females and 43% of yearling males,

occasionally make short-term exploratory movements of up to 20 km before returning to their home ranges (Skuldt et al. 2008).

Home range:

In some northern climates, deer may be migratory and have separate home ranges for summer and winter (Nelson and Mech 1984), but in southern regions deer typically are nonmigratory and have only a single home range (Hirth 1977). In south-central Wisconsin, which is a similar latitude to my study area, deer have been found to migrate only rarely between summer and winter home ranges (Oyer et al. 2007).

Fawns are highly associated with their mothers on their mother's home range for 10-12 months after birth (Hirth 1977, Nelson and Mech 1984), but both male and female yearlings may be driven away in the spring by their mothers (Hirth 1977). Although female yearlings separate from their mothers at one year of age, they still have occasional contact with their mothers through the summer, and form home ranges that are adjacent to or even substantially overlapping with the home range of their mother (Nelson and Mech 1984). Female yearlings typically rejoin their mothers and siblings in the fall and winter (Hirth 1977, Nelson and Mech 1984). Yearling males typically separate from their mothers at one year of age and begin establishing their own home ranges, although some males hang around their mother's home range a few months longer and do not establish their own ranges until they are 15-17 months old (Nelson and Mech 1984). Male yearlings may increase the size of their home range in the fall to more than double the size of their summer home range (Nelson and Mech 1984). Unlike female yearlings, males typically do not rejoin their mothers in the fall and winter (Hirth 1977).

Home range formation may extend over a 2- to 3- year period, with dispersers continuing to visit their natal ranges and associate somewhat with their mothers for at least the first two years of their lives (Nelson and Mech 1984). Summer ranges occupied by yearlings are used again in subsequent years and become the life-long home range (Nelson and Mech 1984). In northern Minnesota, adult summer ranges average 83 ha for females and 319 ha for males (Nelson and Mech 1984). Average adult home range for male deer in mesquite-dominated shrubland habitat in Texas is 207-226 ha (Webb et al. 2007).

Population Genetic Structure and Landscape Genetics

Aspects of deer ecology, especially male-biased dispersal, female philopatry, and home range formation, shape spatial genetic structure of deer populations (Purdue et al. 2000, Comer et al. 2005). Specifically, genetic similarity between deer is expected to be greater for individuals or groups that are spatially closer and to decline as the geographic distance between them increases, a relationship that is known as genetic isolation by distance (Comer et al. 2005, Blanchong et al. 2006). A significant pattern of genetic isolation by distance has been demonstrated between groups of deer on the coastal plains of South Carolina and Georgia (Purdue et al. 2000) and also at the level of individual deer in south-central Wisconsin (Grear et al. 2010) and on a densely forested research park in South Carolina (Comer et al. 2005). Limited female dispersal and the formation of adult home ranges near female relatives results in matrilineal groups that are expected to be genetically related and aggregated in space (Mathews and Porter 1993, Aycrigg and Porter 1997, Nelson and Mech 1999). The higher localized aggregation of related females than males leads to genetic

structure that is female-biased, with females exhibiting greater genetic variability between sites (and more similarity within sites) at a smaller spatial scale than for males (Wang and Schreiber 2001). Male-biased dispersal is expected to maintain gene flow and population connectivity (Nelson 1993) and with this increased dispersal (relative to females) there is increasing genetic homogenization across space and resulting weaker population genetic structure for males than for females (Purdue et al. 2000). Thus, because deer ecology can affect population genetic structure, studying population genetic structure can lead to indirect information about aspects of deer ecology that affect populations.

Because the landscape in which deer live can affect aspects of deer ecology such as dispersal and home range formation, patterns of population genetic structure are expected to be affected by landscape features. Therefore, I chose to use a landscape genetics approach to characterize white-tailed deer population genetic structure in southwestern Wisconsin and northeastern Iowa. Landscape genetics is a combination of molecular population genetics and landscape ecology that explicitly incorporates the effect of landscape features on population genetic structure by looking for correlations between landscape features and genetic patterns (Manel et al. 2003). Major categories of landscape genetics research include quantifying the influence of landscape variables on genetic variation, identifying movement corridors and source-sink dynamics, identifying landscape features that serve as barriers to gene flow, and understanding spatial ecological processes (Storfer et al. 2007). A landscape genetics approach is the tool of choice for many modern-day studies of population genetic structure and the influences of landscape features on gene flow in a wide variety of species. For example, a landscape genetics approach was used to test for differences between spatial patterns of genetic differentiation in American martens (*Martes americana*) in forested and

harvested habitats, and modeling of landscape resistance to marten dispersal was found to explain genetic differentiation better than mere spatial distance between martens (Broquet et al. 2006). Past and present landcover types and habitat connectivity have been used to explain current patterns of population genetic structure of bush-crickets (*Metrioptera roeseli*) in rural landscapes of Germany (Holzhauer et al. 2006). The locations of habitat bioregions have been used to explain patterns of genetic clustering in coyotes (*Canis latrans*) that result from disproportionate dispersal to habitat types similar to an individual's natal habitat (Sacks et al. 2004). Population genetic structure of red deer (*Cervus elaphus*) has been investigated using a landscape genetics approach that assessed the correlation of natural and human-made landscape features with genetic differentiation (Perez-Espona et al. 2008). The influence of human-made features, such as large highways, on connectivity and genetic diversity of desert bighorn sheep (*Ovis canadensis nelsoni*) has also been assessed using a landscape genetic approach (Epps et al. 2005).

Implications for Management

The information gained from my study objectives of 1. characterizing the degree of genetic connectivity between deer populations in Iowa and Wisconsin to identify factors influencing the risk of CWD entering Iowa from Wisconsin, and 2. characterizing female population genetic structure of white-tailed deer in northeastern Iowa to understand effects of the landscape on that genetic structure, could assist the IDNR in development of future management for deer populations in Iowa. Specifically, information regarding white-tailed deer population genetic structure within northeastern Iowa could be used as a basis for fine-

tuning the IDNR Wildlife and Law Enforcement Bureau's Chronic Wasting Disease Response Plan (hereafter, The Plan; (IDNR 2009). The Plan makes recommendations for sample sizes for yearly surveillance of CWD, with higher sample sizes for northeastern Iowa counties along the Mississippi River than for the rest of the state due to the presence of CWD in Wisconsin and Illinois, such as is currently conducted (see Fig. 3 for an example of sample distribution). My study results on the degree of genetic structure found in my study area, and what it suggests about movement of deer across the Mississippi River and within northeastern Iowa, could be used to fine-tune the number of CWD surveillance samples collected yearly by the IDNR and identify which counties might be the most important for higher-density sampling efforts. The Plan also details proposed actions to be taken if CWD is detected in Iowa, including a five-mile (8.0 km) radius surveillance zone of increased sampling around the location of any CWD-positive case (either a free-ranging deer or an individual from a captive cervid facility), and should additional cases be detected from the increased surveillance, a five-mile radius zone where depopulation of all free-ranging cervids is planned in an effort to eradicate the disease. My data on the degree of genetic structure found in northeastern Iowa deer could be helpful in evaluating whether the five-mile radius surveillance and depopulation zones laid out in The Plan are likely to be adequate for containing a CWD outbreak or whether the radius should be adjusted.

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CHAPTER 2. POPULATION GENETIC STRUCTURE OF WHITE-TAILED DEER IN IOWA AND WISCONSIN: UNDERSTANDING RISK OF CHRONIC WASTING DISEASE SPREAD

A paper modified from a manuscript to be submitted to the Journal of Wildlife Management

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Introduction

Characterizing population genetic structure and dispersal across landscapes is critical to understanding a number of biological processes, including the spread of diseases. The evolutionary processes that create genetic differentiation between populations are generally affected by the geographic context of those populations, whether it is simply the distance between populations affecting the degree of differentiation (Diniz-Filho et al. 2009), or some aspect of the connectivity of a species' habitat influencing which populations exchange more individuals than other populations (Broquet et al. 2006). The degree of connectedness between populations can have impacts on the genetic diversity of isolated populations. Separation of populations by landscape barriers can cause such rapid declines in genetic diversity that local population extinction becomes a concern (Epps et al. 2005), and thus it is generally considered beneficial to have genetic exchange between populations. However, a high degree of connectivity between populations can also be problematic, especially when the transmission of diseases between infected and uninfected populations is considered.

Assessing the correlation of population genetic structure and landscape features can lead to identification of barriers to dispersal that may affect the future spread of wildlife diseases (Cullingham et al. 2009).

Identifying factors that influence the spatial spread of wildlife diseases is crucial for designing effective surveillance programs and appropriate management strategies. For example, information on the effect of rivers on genetic connectivity and dispersal of raccoons can be used to direct management of rabies by determining the size and location of buffer zones in which to focus wildlife vaccination programs (Cullingham et al. 2009). Knowledge of the scale of population genetic structure and limited effect of landscape attributes on gray fox dispersal has been used to define the appropriate width for rabies vaccination buffer zones in Texas (Deyoung et al. 2009). The spatial scale at which differences occur in mean relatedness of bovine tuberculosis-infected and uninfected white-tailed deer can be used to understand the potential role of deer social groups in disease transmission and aid in management decisions (Blanchong et al. 2007). The potential introduction of chronic wasting disease (CWD), a fatal neurodegenerative disease of cervids, to Iowa is of significant management concern because it is found in several bordering states including Wisconsin, where it was first detected in free-ranging white-tailed deer (*Odocoileus virginianus*) harvested in 2001 from the south-central region of the state (Joly et al. 2003).

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) caused by a proteinaceous infectious agent referred to as a prion. It is related to other TSEs that affect ruminants, such as bovine spongiform encephalopathy (BSE, “mad cow disease”) and scrapie of sheep and goats, that are of significant economic concern, and in the case of BSE, of considerable public health concern as well (Williams et al. 2002). Several cervid species

are known to be affected by CWD including white-tailed deer, mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) (Miller et al. 2000), and moose (*Alces alces*) (Kreeger et al. 2006). It appears that the vast majority of transmission is horizontal, with only about 3.4% vertical transmission between mother and fawn in mule deer (Miller et al. 2000). In white-tailed deer in the Midwest, males have a higher probability of becoming infected than do females, and disease prevalence increases with age, more so for males than females (Grear et al. 2006). Because CWD is always fatal (Sigurdson 2008), it could have serious impacts on the viability of deer populations, and some modeling has suggested that CWD epidemics could drive deer populations to extinction in the locality of the epidemic (Miller et al. 2000). The maintenance of viable deer populations is of great concern to state wildlife agencies because deer hunting provides a major source of revenue in the form of deer hunting license sales for agencies such as the Iowa Department of Natural Resources (IDNR), and also stimulates the local economy through other purchases by hunters (Stone 2003).

Chronic wasting disease was first identified in mule deer in Colorado during the 1960s (Williams et al. 2002), but it has spread geographically over the past several decades and has now been found in free-ranging populations of cervids in eleven U.S. states and two Canadian provinces (CDC 2010). The current distribution of the disease appears to be partly related to natural movements of free-ranging deer and elk, and partly a result of commercial movement of infected captive animals from one geographic region to another (Belay et al. 2004). Indeed, it is considered likely that the movement of an infected captive cervid into Wisconsin was the initial cause of the detection of disease east of the Mississippi River (Joly et al. 2003).

Since the discovery of CWD in free-ranging white-tailed deer in Wisconsin in 2001, it has also been found in the Midwest in captive Wisconsin cervids, free-ranging Illinois white-tailed deer, and captive elk in Minnesota (Joly et al. 2003). The Wisconsin Department of Natural Resources (WDNR) has been testing deer for CWD since 1999 (Joly et al. 2003) and two deer testing positive for CWD have been found in a Wisconsin county (Grant) bordering Iowa. To date, there have not been any CWD-infected cervids identified in the state of Iowa, despite rigorous testing of approximately 4,000 harvested animals per year since 2006 (IDNR 2010). There is considerable interest in understanding the potential for CWD spread to Iowa through the natural movement of free-ranging deer from Wisconsin, given Iowa's close proximity to current infection zones but separation from Wisconsin by the Mississippi River. However, little is known about the dynamics of deer dispersal, and attendant risk of CWD-spread, between Wisconsin and Iowa across the Mississippi River.

White-tailed deer are found across much of North America and occupy a wide variety of habitats, from deciduous and coniferous forests to more open ranges with broad plains or savannas (Hirth 1977). Forests and forest edges provide important cover for deer (Halls 1984) and in the more open ranges, brushy draws are important for cover (Hirth 1977). Males are traditionally viewed as the dispersing sex and females are typically philopatric, establishing adult home ranges in their natal area (Hawkins and Klimstra 1970, Nelson and Mech 1984). Male dispersal rates vary among studies in different landscapes and range from 46 – 90%, and average dispersal distances range from 7 – 41 km (Hawkins and Klimstra 1970, Nelson and Mech 1984, Nixon et al. 1991, Long et al. 2005, Webb et al. 2007). The percentage of females that disperse varies dramatically among studies and appears to be influenced by the amount of forested habitat, ranging from 3% in forested landscapes to 49%

in highly agricultural areas (Hawkins and Klimstra 1970, Nixon et al. 2007, Skuldt et al. 2008), with average dispersal distances ranging from 4.5 km in forested areas to 41 km in agricultural regions.

Aspects of deer ecology, especially male-biased dispersal, female philopatry, and home range formation, shape spatial genetic structure of deer populations (Purdue et al. 2000, Comer et al. 2005). Specifically, genetic similarity between deer is expected to be greater for individuals or groups that are spatially closer and to decline as the geographic distance between them increases, a relationship that is known as genetic isolation by distance (Comer et al. 2005, Blanchong et al. 2006). Limited female dispersal and the formation of adult home ranges near female relatives results in matrilineal groups that are expected to be genetically related and aggregated in space (Mathews and Porter 1993, Aycrigg and Porter 1997, Nelson and Mech 1999). The higher localized aggregation of related females than males leads to genetic structure that is female-biased, with females exhibiting greater genetic variability between sites at a smaller spatial scale than for males (Wang and Schreiber 2001). Therefore it is reasonable to assume that a female is more closely related to and representative of the genetics of the deer in the immediate area of her home range than an adult male would be. Male-biased dispersal is expected to maintain gene flow and population connectivity (Nelson 1993) and with this increased dispersal (relative to females) there is increasing genetic homogenization across space, resulting in weaker population genetic structure for males than for females (Purdue et al. 2000).

We conducted a study of the population genetic structure of white-tailed deer in southwestern Wisconsin and northeastern Iowa as a step toward understanding risk of CWD-infected deer crossing the Mississippi River, entering Iowa, and spreading CWD to

populations where the disease is not known to be present. There have been and are currently other landscape genetic studies of white-tailed deer in nearby areas. Landscape genetics, a combination of molecular population genetics and landscape ecology, is the tool of choice for many modern-day studies of population genetic structure and the influences of landscape features on gene flow (Manel et al. 2003, Sacks et al. 2004, Perez-Espona et al. 2008). Unlike many previous genetic methods, landscape genetic techniques can work without first having to predefine discrete populations (Manel et al. 2003, Schwartz and McKelvey 2009) and can instead define the numbers and locations of populations based on the genetic data themselves (Sacks et al. 2004, Perez-Espona et al. 2008). A previous landscape genetic study in Wisconsin found that the population genetic structure of white-tailed deer is influenced by landscape features such as rivers, which may act as a barrier to movement. This might result in substantially lower probabilities of CWD introduction via movement of infected deer to populations that are separated by rivers from highly infected areas (Blanchong et al. 2008).

Our main objective was to characterize the degree of genetic connectivity between deer populations in Iowa and Wisconsin to identify factors influencing the risk of CWD entering Iowa through the natural movement of free-ranging deer from Wisconsin. We hypothesized that the Mississippi River would restrict deer gene flow between the states and thus spread of CWD by dispersing, infected individuals. To determine the influence of the Mississippi River on deer population genetic structure, we identified the number and location of genetically discrete populations and assessed whether there were patterns of genetic isolation by distance at two different spatial scales. We also tested for evidence of sex-biased dispersal across the Mississippi River. We hypothesized that dispersal would be male-biased, which is particularly important for understanding potential for CWD spread because males

are the sex that has the higher prevalence of CWD.

Study Area

The study area consisted of four northern Iowa counties and three western Wisconsin counties along the Mississippi River (Fig. 1). Although Delaware County in Iowa does not directly touch the river, it has been included because the northeast corner of the county reaches within about 12 km of the river, and deer population genetic structure and risk of infection with CWD in this county could be influenced by dispersing individuals from Wisconsin. The study area was 13,589 km^2, with counties ranging in size from 1,500 to 3,065 km^2. Forests, which are primarily composed of deciduous trees, cover 41% of the landscape and are somewhat fragmented. Grasslands represent 39% of the area, and row-crop agriculture characterized by corn and soybeans covers 15% of the landscape. Human settlements are relatively minimal, with only 2% of the land in cities and roads. The study area falls on the southwestern edge of the “Driftless Area”, so called because it was not covered by glacial drifts during the last ice age, and has a relatively dissected topography characterized by a diversity of microhabitats and higher plant diversity than other areas of Iowa (Pusateri et al. 1993).

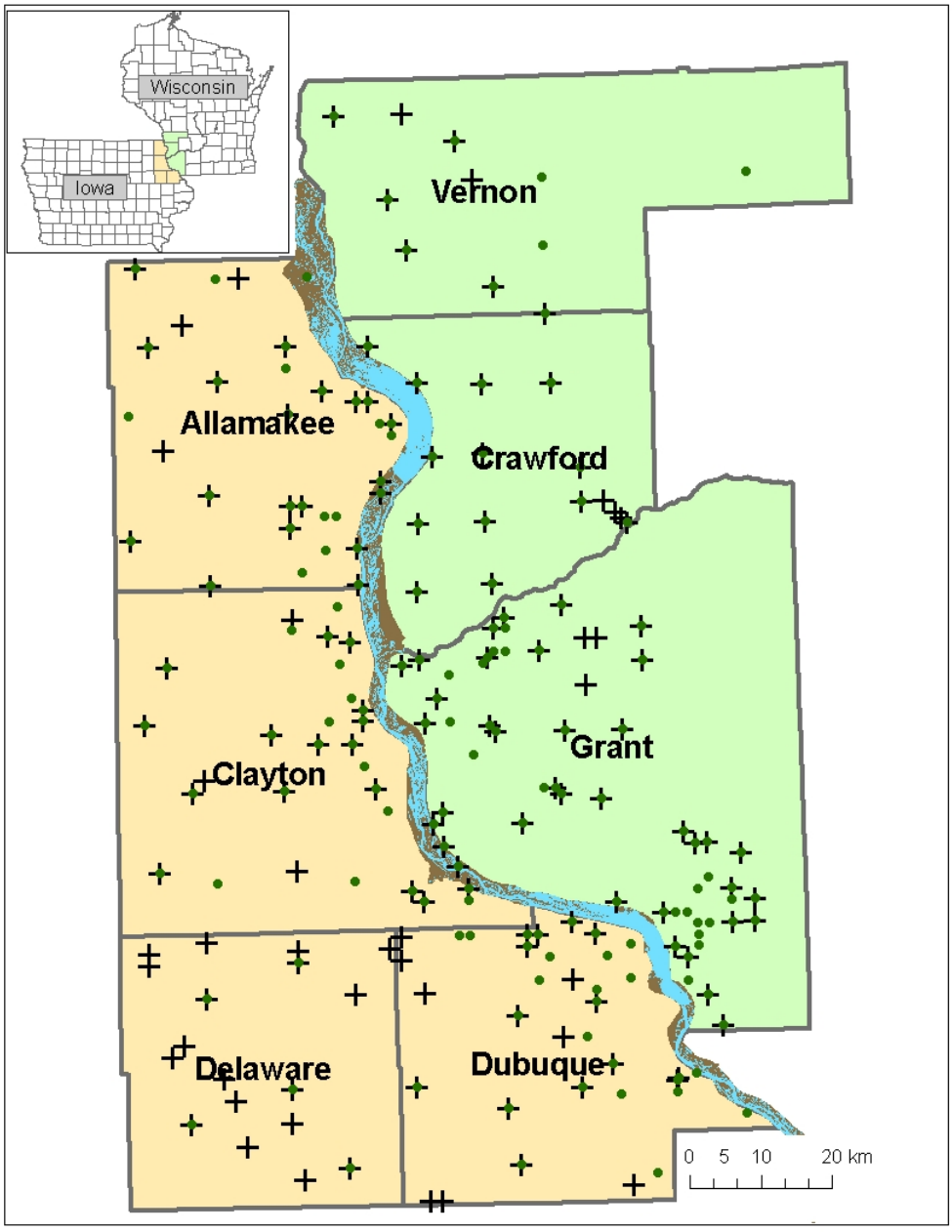

Figure 1. Study area comprising seven counties, features of the Mississippi River channel (water = blue, land = brown), and the harvest location of deer samples used for microsatellite data (green dots) and mtDNA data (black crosses).

The stretch of Mississippi River included in our study area has three dams that create pools, upstream of which the channel narrows and islands and side channels become more

common (Fig. 1). This creates considerable heterogeneity along the river in the width of the waterways across which a deer would have to swim. Since 1866, the U.S. Army Corps of Engineers has been modifying the river channel with dikes, dams, and locks to create a navigable channel (Rasmussen and Pitlo Jr. 2004). The navigation channel was originally maintained at 3 feet deep, was expanded to 4.5 feet in 1878, and since the 1930s the stretch from Cairo, Illinois, to St. Paul, Minnesota, has been maintained at 9 feet. In order to create the deeper navigation channel, many wing dikes have been constructed which have resulted in the loss of many side channels from natural sedimentation, as well as human filling and dumping of dredge spoils (Rasmussen and Pitlo Jr. 2004).

Materials and Methods

Sampling:

Samples of deer tissue (lymph nodes and/or brain stem) collected by the IDNR and WDNR during 2006 for CWD testing were procured for genetic analysis. Most of these were from hunter-harvested deer during the fall hunting season, but a few Iowa samples were from roadkill or deer that were targeted for CWD-sampling because of unusual behavior or exhibition of potential CWD symptoms.

During collection of tissue samples, spatial information on the location of harvest was collected by the Iowa and Wisconsin DNRs. These data varied in spatial resolution from exact Universal Transverse Mercator (UTM) coordinates to townships. The majority (93%) of samples from Iowa had section-level spatial resolution, while those from Wisconsin had spatial resolution varying from the level of a quarter section (30%) to a section (14%) to a

township (53%). Most of the samples from Wisconsin that had only township level spatial information came from the northern half of the Wisconsin study area (Vernon and Crawford counties, Fig. 1) and were not ideal for conducting analyses at a fine spatial scale.

From the available samples, a sample of individuals in each county was chosen, with as even geographic coverage as possible. We chose this sampling approach because there are no obvious landscape features (other than the Mississippi River) that might create deer population boundaries by which we could predetermine the locations of populations to select samples from. The even distribution of samples across the landscape was intended to give us the best basis for characterizing population genetic structure in the presumably continuously distributed population across our study area, and is the most appropriate sampling scheme for use in clustering analyses (Schwartz and McKelvey 2009). Female deer were chosen for genetic analyses because females are the philopatric sex in white-tailed deer and are expected to give a more representative picture of historical genetic composition of deer populations in the local area where an individual was harvested than would males.

Laboratory methodology:

In order to have a strong basis for characterizing deer population genetic structure, we used both nuclear microsatellite markers and mitochondrial DNA (mtDNA) sequences. Microsatellites, stretches of presumably neutral DNA with short tandem repeat units of one to several nucleotides, are ideal for population genetic studies because they are highly variable with a potentially large number of alleles per locus and generally occur in non-coding regions where they are presumed to evolve neutrally (Ellegren 2004). We chose twelve nuclear biparentally inherited microsatellites (all dinucleotide repeat units) previously demonstrated

to be polymorphic in white-tailed deer (Dewoody et al. 1995, Anderson et al. 2002, Blanchong et al. 2006, Blanchong et al. 2008). Many of the same microsatellite loci are being used in other studies of white-tailed deer genetics in Wisconsin and Illinois. Similarly, the region of mtDNA sequenced is the same as that used in studies in Wisconsin. Mitochondrial DNA, which is only inherited maternally, is especially useful to characterize population genetic structure because it tends to show more differentiation than microsatellites and is expected to give an even stronger signature of female population genetic structure. We sequenced the mtDNA control region (d-loop), a stretch of non-coding DNA that is highly variable (Grear et al. 2010) and therefore useful for intraspecific studies.

DNA for genetic analyses was extracted with a DNeasy® Blood & Tissue kit (Qiagen, Valencia, CA, USA), using lymph node tissue (whenever possible) because it appeared from our early lab work that lymph node tissue yielded higher concentrations and quality of DNA than brain stem tissue. Primers for amplification of the microsatellites and desired section of mtDNA were synthesized by the Iowa State University Office of Biotechnology's DNA Facility (hereafter, ISU DNA Facility).

Microsatellites: For microsatellite genotyping (n = 249 deer) we used the following loci: BM1225, BM4107, BM4208 (Bishop et al. 1994), OarFCB193 (Buchanan and Crawford 1993), Cervid 1, Cervid 2 (Dewoody et al. 1995), IGF-1 (Kirkpatrick 1992), OBCAM (Moore et al. 1992), RT7, RT9, RT23, and RT27 (Wilson et al. 1997). The polymerase chain reaction (PCR) was used to amplify the microsatellite loci, which were combined into four multiplexed reactions of three loci each: 1) OarFCB193, BM4208, OBCAM; 2) BM4107, Cervid 1, BM1225; 3) RT9, RT27, Cervid 2; and 4) IGF-1, RT23, RT7. Forward primers

were fluorescently labeled with either 6-FAM or HEX (Applied Biosystems, Foster City, CA, USA) for visualization during genotyping. Reactions were conducted in 12 ul volumes (2.0 µl of 20 ng/µl DNA, 0.07 – 0.23 µl each of 10 µM forward and reverse primers for three microsatellites per reaction, 5.0 µl of 2X Multiplex PCR Master Mix [Qiagen], and sterile DNA grade H_2O to make up remaining volume). Amplification was achieved in an Eppendorf Mastercycler epgradient under the following conditions: initial activation 95°C for 15 min, followed by 10 cycles of primer-specific amplification with 94°C denaturation for 30 sec, 90 sec anneal starting at 63°C and stepping down to 57°C through subsequent cycles, 72°C extension for 60 sec, followed by 21 cycles of universal amplification with 94°C denaturation for 30 sec, 57°C anneal for 90 sec, 72°C extension for 60sec, and a final extension at 60°C for 30 min. A small sample of PCR product was visualized on agarose gel to confirm successful amplification. PCR products were genotyped by the ISU DNA Facility on an ABI 3100 Genetic Analyzer and results scored for allele length (base pairs) using Peak Scanner v1.0 (Applied Biosystems, Foster City, CA, USA). Quality control was accomplished in a number of ways, including running a few samples from deer whose genotypes were known (based on previous work in other labs) in each PCR and 96-well genotyping plate, having at least two experienced individuals independently score genotyping results and then compare scores, re-amplifying and re-genotyping all samples for which a genotype could not be agreed upon, and re-running four randomly chosen samples per genotyping plate to confirm consistency of results.

Mitochondrial DNA: For the mtDNA (n = 173 deer), we sequenced a 699 base-pair portion of

the mtDNA control region (d-loop) using PCR primers (forward: 5'-TCT CCC TAA GAC TCA AGG AAG -3', reverse: 5'- GTC ATT AGT CCA TCG AGA TGT C-3') developed by Miyamoto et al. (1990; Genbank Accession M35874). The control region was amplified in 12.5 µl volumes (2.5 µl DNA at a concentration of 20 ng/µl, 0.95 µl of 10X PCR buffer [Denville Scientific Inc., Metuchen, NJ, USA], 1.0 µl of 10 mM dNTPs, 1.0 µl each of 10 µM forward and reverse primers, 1.45 µl of 25 mM $MgCl_2$, 0.17 µl of Hot-Start Taq [Denville Scientific Inc., Metuchen, NJ, USA], 4.43 µl sterile DNA grade H_2O). Amplification conditions were: initial activation 95°C for 5 min, followed by 25 cycles with 94°C denaturation for 30 sec, 52.5°C anneal for 30 sec, and 68°C extension for 30 sec, with a final extension at 68°C for 3 min. A small sample of the PCR product was visualized on agarose gel to confirm successful amplification, after which a portion of the PCR product was purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) following the manufacturer's protocol to remove excess PCR reagents.

The sequencing reaction was carried out by the ISU DNA Facility using primer and purified template provided by us. We used the PCR reverse primer as the sequencing primer, as we had difficulties with the nested sequencing primer developed by Miyamoto et al. (1990) and found the PCR reverse primer to produce more reliable results. This substitution of primers did not affect the section of the sequence we used for analyses or its comparability with studies in other states. Following the sequencing reaction, the sequence was run on the ISU DNA Facility's ABI 3730xl DNA Analyzer. The resulting sequence data (traces) were visualized in Sequence Scanner v1.0 (Applied Biosystems, Foster City, CA, USA) and checked visually for correct base-calls. Sequences were then imported into MEGA version 4 (Kumar et al. 2008) along with known haplotype sequences from previous work in Wisconsin

(Grear et al. 2010) and aligned using the ClustalW algorithm (Thompson et al. 1994). The haplotype (representing all variation leading to a unique sequence) of a sample was then determined by process of elimination when comparing it to all known haplotypes. If a sample had a unique, previously unseen sequence that did not match any of the known haplotypes, we double-checked the sequence for correct base calls and then assigned it as a new haplotype. Quality control of the sequences was accomplished by including a blank sample (negative control) on each 96-well plate of samples submitted for sequencing. Samples that did not produce clean sequence that could be scored reliably on the first run were re-amplified and sequenced again. Correct assignment of sequences to haplotypes was checked independently by having a subset (10%) of samples assigned haplotypes by a second person and comparing these haplotype assignments to haplotypes assigned the first time.

Population genetic analyses:

Summary statistics: We tested each of our microsatellite loci for the presence of null alleles (alleles that failed to amplify during PCR), large allele dropout caused by weak amplification of longer alleles, and stutter scoring errors from mis-scoring of stutter peaks rather than allele peaks using Micro-Checker ver. 2.2.3 (Van Oosterhout et al. 2004). Hardy-Weinberg equilibrium (HWE) and linkage equilibrium are assumptions of many genetic analyses, so we tested for departures from equilibrium using a Markov chain approach in Genepop on the Web ver. 4.0.10 (Raymond and Rousset 1995) with all samples combined and also samples from Iowa and Wisconsin tested separately. The run of the Markov chain algorithm started with a dememorization period long enough that the state of the Markov chain at the end is independent of the initial state, then additional runs of the chain were divided into batches

each a certain number of iterations long, and the proportion of iterations that result in a data configuration more extreme than the observed data were used to calculate the probability of observing our data. Our Markov chains consisted of 1,000 dememorization steps, 200 batches, and 2,000 iterations. A sequential Bonferroni correction for multiple tests (Rice 1989) was applied to the results of the linkage equilibrium tests because of the large number of comparisons involved. We also used Genepop to calculate F_{IS} (the inbreeding coefficient of individuals within subpopulations) and observed and expected heterozygosities for microsatellites, as well as gene diversity for both types of markers.

Population clustering: We used two different methods of determining the number of populations represented by our data: maximum likelihood and Bayesian analysis. The maximum likelihood approach was implemented using the program PartitionML (Castric et al. 2002) which calculates the likelihood of an individual's multilocus genotype and uses the product of the likelihoods of individuals in each cluster to determine the likelihood of partitioning the data into *K* clusters. Successive increases in *K* were tested until there was no improvement in the fit of the model based on a likelihood ratio test (alpha < 0.05) that penalized the addition of extra model parameters. Analyses with this program were conducted only for the microsatellite data because it cannot handle haplotype data.

We tried both non-spatial and spatially-explicit Bayesian clustering approaches for identifying populations using both the microsatellite data set and the haplotype data set. The non-spatial approach was conducted in Structure ver. 2.3.1 (Pritchard et al. 2000, Falush et al. 2003), a program that assumes a model with *K* populations and probabilistically assigns individuals to populations (or jointly to multiple populations if the admixture model is used

and their genotypes indicated admixture) in such a way as to minimized deviations from Hardy-Weinberg equilibrium within populations (Pritchard et al. 2009). We implemented the admixture model, allowing the degree of admixture (α) to be different for each population, and set allele frequencies (λ) to be correlated between populations because, for a highly mobile species such as deer, we expect enough connectivity between populations to maintain similar allele frequencies in different populations. Ten independent runs were conducted for each K = 1-5, with five populations being more than we would reasonably expect to find in our study area, using a Markov chain Monte Carlo (MCMC) approach with a burn-in period of 100,000 followed by 1,500,000 iterations. To determine the most likely number of populations, we plotted the log-likelihood values for each run against K following the graphical method of DeYoung et al. (2009), where the best result (the most likely number of populations) is indicated by the highest value of the log-likelihood.

Our spatially-explicit Bayesian clustering analyses were conducted in the Geneland ver. 3.1.5 (Guillot et al. 2005b) package of the statistical program R ver. 2.10.0 (Ihaka and Gentleman 1996). Geneland uses spatial coordinate information in addition to the genetic data to make inferences about the number of populations represented by the data set and the spatial location of boundaries between these populations. A MCMC technique is used to iteratively determine the most likely number of populations (K), with all values of K considered to be *a priori* equally likely, and cluster individuals into populations such that each population is approximately in Hardy-Weinberg and linkage equilibrium (Guillot et al. 2005a). We incorporated an uncertainty on the spatial coordinates of 1609 m (1 mile) because most of the spatial data were collected at the level of a section (1 mi^2). We ran the model five times allowing K to vary between minimum K = 1 and maximum K = 10. The maximum K

was set to ten because we did not want to constrain iterations of the model to anything lower, although ten populations was much higher than what we expected might be reasonable for our study area. We conducted 500,000 MCMC iterations, with a thinning of 500, allele frequencies uncorrelated between populations, and all other values at their default settings. We chose to use the uncorrelated allele frequency model because it is more robust than the correlated frequency model even for data sets simulated with allele frequencies correlated (Guillot et al. 2005a). The most likely number of inferred populations was determined by looking at the modal *K* in a histogram of all *K* produced by the MCMC iterations and by counting the number of populations in the map output produced by Geneland. We then conducted twenty runs with *K* fixed at the inferred number of populations and all other parameters the same as above. The mean probability of population membership for each individual was calculated across the twenty runs and these were plotted in ArcGIS (ESRI, Redlands, CA, USA) to visualize individual population membership and the location of population divisions in comparison to the landscape.

Genetic differentiation: We explored the effect of the Mississippi River on genetic differentiation in the study area at the spatial scales of both the state (Iowa and Wisconsin) and the county. The county level was selected because IDNR manages deer and sets deer hunting quotas at the level of the county (Litchfield 2008), which may have an effect on deer population structure. A common metric of genetic differentiation is F_{ST}, the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance (Wright 1951). Because we used genetic markers with two different modes of inheritance, we chose to use Φ_{PT}, an analog of F_{ST} that facilitates comparisons between codominant and

haploid data by suppressing the within-genotype variation of codominant data to make it more similar to haplotype data (Peakall and Smouse 2006). We computed Φ_{PT} for both the microsatellite and mtDNA data sets by conducting an Analysis of Molecular Variance (AMOVA), which partitions the total genetic variation in the data into the variation among groups and the variation within groups, in GenAlEx ver. 6.3 (Peakall and Smouse 2006). We computed comparisons between the two states and between all possible pairs of counties, both those pairs on opposite sides of the Mississippi River from each other and those on the same side. A sequential Bonferroni correction for multiple comparisons was applied to the estimates of pairwise Φ_{PT} between counties.

Isolation by distance: In a large-bodied mobile species, such as white-tailed deer, there may not be any sharp boundaries creating discrete genetic clusters or populations, but that does not necessarily mean that there is a complete lack of genetic structure. The mating of proximal individuals could create a genetic gradient (i.e., isolation by distance) across the landscape with individuals that are spatially closer to each other being more closely related than those that are further apart. We conducted a number of tests to look for patterns of increasing genetic difference with increasing geographic distance or as a function of 'ecological' distance. Some analyses were conducted at the level of the county and some were at the level of the individual to explore fine-scale population structure.

For the county-level tests of isolation by distance, we used Mantel tests (Mantel 1967), which performed a linear regression of a y-matrix of pairwise genetic distance between counties against an x-matrix of the corresponding pairwise geographic distances. The Mantel tests were performed in GenAlEx ver. 6.3 (Peakall and Smouse 2006) using 999

permutations to test for significance of the relationship. We used two different measures of genetic distance: the degree of genetic differentiation (Φ_{PT}) between counties (calculated as above); and Cavalli-Sforza and Edward's chord distance (Cavalli-Sforza and Edwards 1967), which we calculated using the GenDist program of the Phylip ver. 3.69 suite of programs (Felsenstein 1989), that assumes genetic differences arise due to genetic drift and does not assume equal or constant population size (Cavalli-Sforza and Edwards 1967). The geographic distance between counties was calculated in the suite GeneticStudio build 131 (Dyer 2009) using the program Geno, Ln-transformed, and transferred to GenAlEx for use in the Mantel tests. For the microsatellite data we removed Delaware (n = 5 deer) and Vernon (n = 8 deer) counties from the analysis because we did not feel there were enough samples from those counties to accurately represent the genetic variation or allele frequencies, and for the mtDNA data we removed only Vernon county (n = 7 deer).

Geographic distance may not be the only factor that influences dispersal of deer and degree of genetic relationship among deer in different areas. There may be costs, such as swimming across the Mississippi River, that influence the 'effective' or 'ecological' distance between deer and may explain spatial genetic patterns better than geographic distance. To test for the effect of the Mississippi River, we created a matrix of pairwise ecological distances between counties. A distance of zero (0) was used for pairs of counties that were on the same side of the river and a distance of one (1) was used for pairs of counties that were separated from each other by the river and which we hypothesized were more costly to move between. We then conducted Mantel tests in GenAlEx of the ecological distance against the same two genetic distances used above for comparisons with geographic distance.

To test for patterns of isolation by distance at the individual level we used the genetic

relationship coefficient Moran's I (Epperson and Li 1996), which can be used to test for spatial autocorrelation – the correlation of spatially adjacent observations with the geographic distance between them – and used to detect departures from spatial randomness (Moran 1950) that indicate patterns such as genetic isolation by distance. We used the program SPAGeDi ver. 1.3a (Hardy and Vekemans 2002) to calculate Moran's I and plotted it against binned inter-individual geographic distances. For all tests, 999 permutations were performed to produce a 95% confidence interval around the null. Distance classes were in intervals of 9654 m (6 miles), equivalent to the size of a township and our coarsest resolution of spatial data.

Sex-biased dispersal: Deer are traditionally considered to exhibit sex-biased dispersal, with males being the primary dispersers and females remaining philopatric (Hawkins and Klimstra 1970). Having genetic markers with two different modes of inheritance (biparentally-inherited microsatellites and matrilineally-inherited mtDNA) allows us to test for evidence of sex-biased dispersal even though we only used samples from a single sex (females). Mitochondrial DNA is expected to show greater population differentiation than nuclear microsatellites because it is haploid and uniparentally inherited, creating an effective gene pool that is one quarter the size of the gene pool for the microsatellites, and making mtDNA more readily affected by genetic drift (Birky et al. 1989). To better understand whether any observed differences between estimates of population differentiation from the two types of genetic markers was solely a result of differences between the inherent characteristics of the markers or contained additional effects resulting from differences in the gene flow, we tested for sex-biased dispersal.

Specifically, the program SPAGeDi ver. 1.3a (Hardy and Vekemans 1999) was used to calculate estimates of F_{ST} for both kinds of markers and standard errors for these by jackknifing across loci. For the mtDNA sequence, the data were input as a series of variable sites, instead of as a single haplotype locus, to create multiple "loci" across which jackknifing could occur. Coding the mtDNA variable sites as loci appropriately represents the variation within our data during the jackknifing process as a single locus (variable site) at a time is left off and the statistic (F_{ST}) is recalculated (Quenouille 1956). The standard errors (SE) for the estimates of F_{ST} were used to create 95% confidence intervals with a width of 1.96 * SE on either side of the estimate. The upper and lower bounds of the confidence interval for the microsatellite F_{ST} [i.e., $F_{ST(nuclear)}$] were translated to the expected F_{ST} for mtDNA using the equation $F_{ST(mitochondrial)} = 4\ F_{ST(nuclear)} / [1 + 3F_{ST(nuclear)}]$ (Crochet 2000) and compared to the confidence intervals of the actual mtDNA F_{ST} to see if there was more differentiation than expected solely due to the difference in the markers. If the confidence interval of the *observed* mtDNA F_{ST} does not overlap with the confidence interval for the mtDNA F_{ST} *expected* under the null hypothesis of equal dispersal (gene flow) by males and females then we can reject the null and conclude that there is sex-biased dispersal (Hamilton and Miller 2002).

Results

Population genetic analyses:

Summary statistics: Of the twelve microsatellite loci, none had evidence of large allele dropout and only Cervid2 exhibited evidence of null alleles and stutter scoring errors.

Therefore, Cervid2 was eliminated from the data set and all further analyses were run with the remaining eleven loci. All samples combined as one group were not significantly out of HWE (P = 0.060) as was the case for Wisconsin (P = 0.432), but the Iowa group showed a significant deviation (P = 0.036), suggesting that the Iowa portion of our study area might not be a single population. No pairs of markers showed significant linkage after the sequential Bonferroni correction, either for the group as a whole or for the states individually. All loci were moderately to highly variable, with between 9 and 17 alleles for the microsatellites (Table 1).

Table 1. Number of alleles (N_A), ratio of observed and 'expected' heterozygosity (H_{obs}/H_{exp}), estimated gene diversity (H_e), and Weir and Cockerham (1984) F_{IS}, for each microsatellite locus and for mtDNA across the study area.

Locus	N_A	H_{obs}/H_{exp}	H_e	F_{IS}
BM1225	11	0.950	0.757	0.052
BM4107	16	0.951	0.813	0.054
BM4208	16	0.913	0.908	0.070
Cervid 1	14	0.982	0.828	0.016
IGF-1	12	1.006	0.649	-0.026
OarFCB193	15	0.988	0.868	0.006
OBCAM	12	0.947	0.871	0.035
RT7	15	0.948	0.887	0.026
RT9	9	0.895	0.819	0.109
RT23	17	0.990	0.919	-0.008
RT27	15	0.976	0.870	0.016
mtDNA	25		0.884	

In the mtDNA, we identified 63 variable sites creating 25 unique haplotypes (Table 1). Gene diversity (H_e), the diversity among individuals within the sample averaged 0.835 for

all microsatellite loci combined and 0.884 for mtDNA. For microsatellites, average F_{IS} (Weir and Cockerham 1984), the inbreeding coefficient of an individual relative to the population, for all loci combined was 0.032.

Population clustering: The likelihood ratio test on the results from PartitionML did not support partitioning of the microsatellite data (and thus the study area) into more than one population. For the test of $K = 1$ versus $K = 2$ the observed chi-square value was 80.03 (critical value = 169.7 for df = 141), indicating that K = 2 populations did not explain the data significantly better than K = 1. All tests for higher values of K were even further from significance.

In analyses using Structure, the highest estimated logarithm of probability of the data [Ln Pr(D|*K*)], averaged across ten runs at each value of *K*, occurred at $K = 1$ for both microsatellite and mtDNA data (Fig. 2), although for the mtDNA data, $K = 5$ also had a very high Ln Pr(D|*K*). Runs with $K > 1$ had generally higher variability than runs with $K = 1$. The mean values of Ln Pr(D|*K*) did not follow an increasing trend with increasing *K* nor show any sign of reaching a plateau, so we did not apply the methods of Evanno et al. (2005) for using rate of change in Ln Pr(D|*K*) to identify the true number of genetic clusters.

Geneland runs for microsatellite data indicated a single cluster as the modal number of populations (Fig. 3a). With the mtDNA data, however, there was sufficient signature of genetic structure for the program to identify two separate clusters (Fig 3b). The probability of population membership for each individual averaged across 20 runs fixed at $K = 2$ was relatively high, with over 90% of the individuals having a > 70% probability of population membership to one of the two clusters, and only a narrow band of individuals with

ambiguous population membership at the interface between the two inferred clusters (Fig. 4).

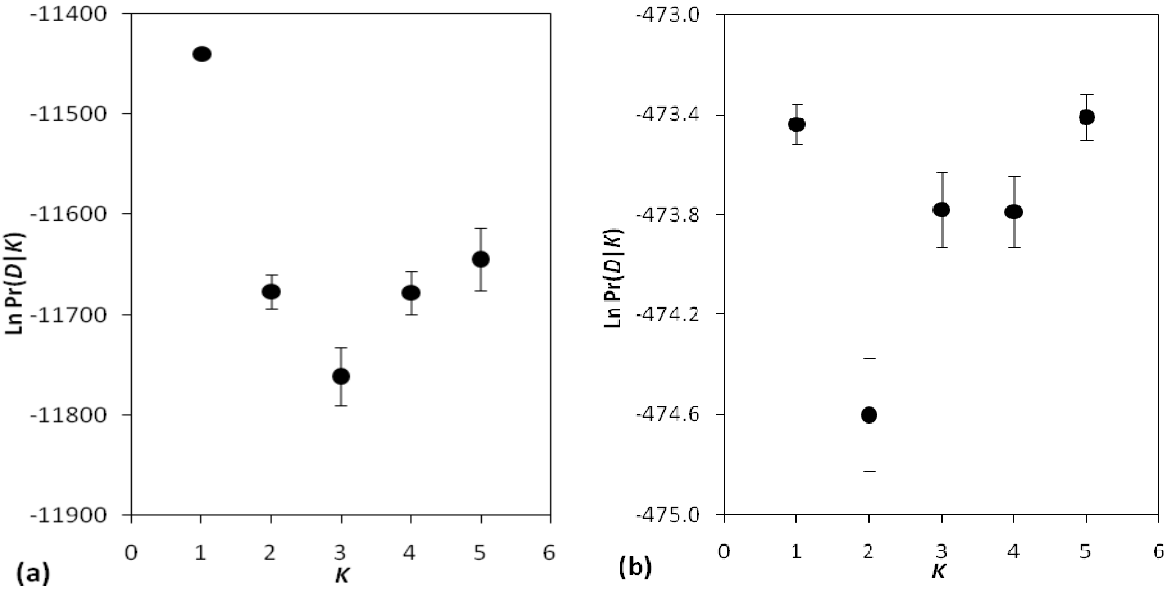

Figure 2. Mean and standard error of estimated log probability of the data[Ln Pr(*D*|*K*)] for each number of populations tested (*K*) in STRUCTURE, for **(a)** microsatellite data and **(b)** mitochondrial DNA data.

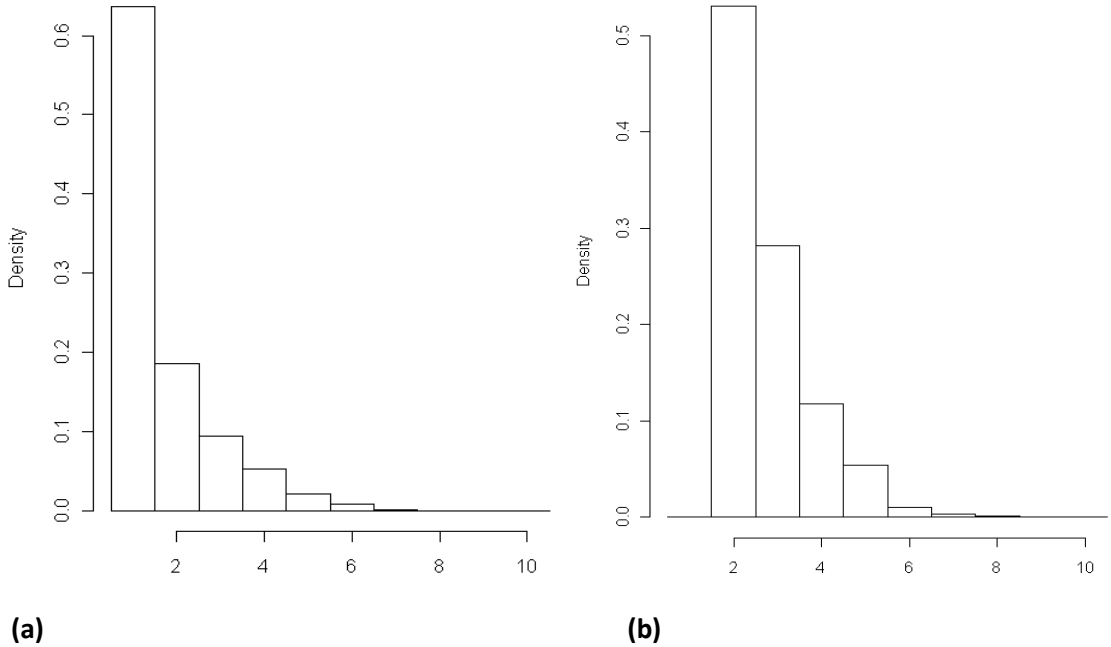

Figure 3. Number of populations along Markov Chain Monte Carlo chain summarized over 500,000 iterations (minus first 50,000 as burnin) run in Geneland for microsatellites **(a)** and mitochondrial DNA **(b)**.

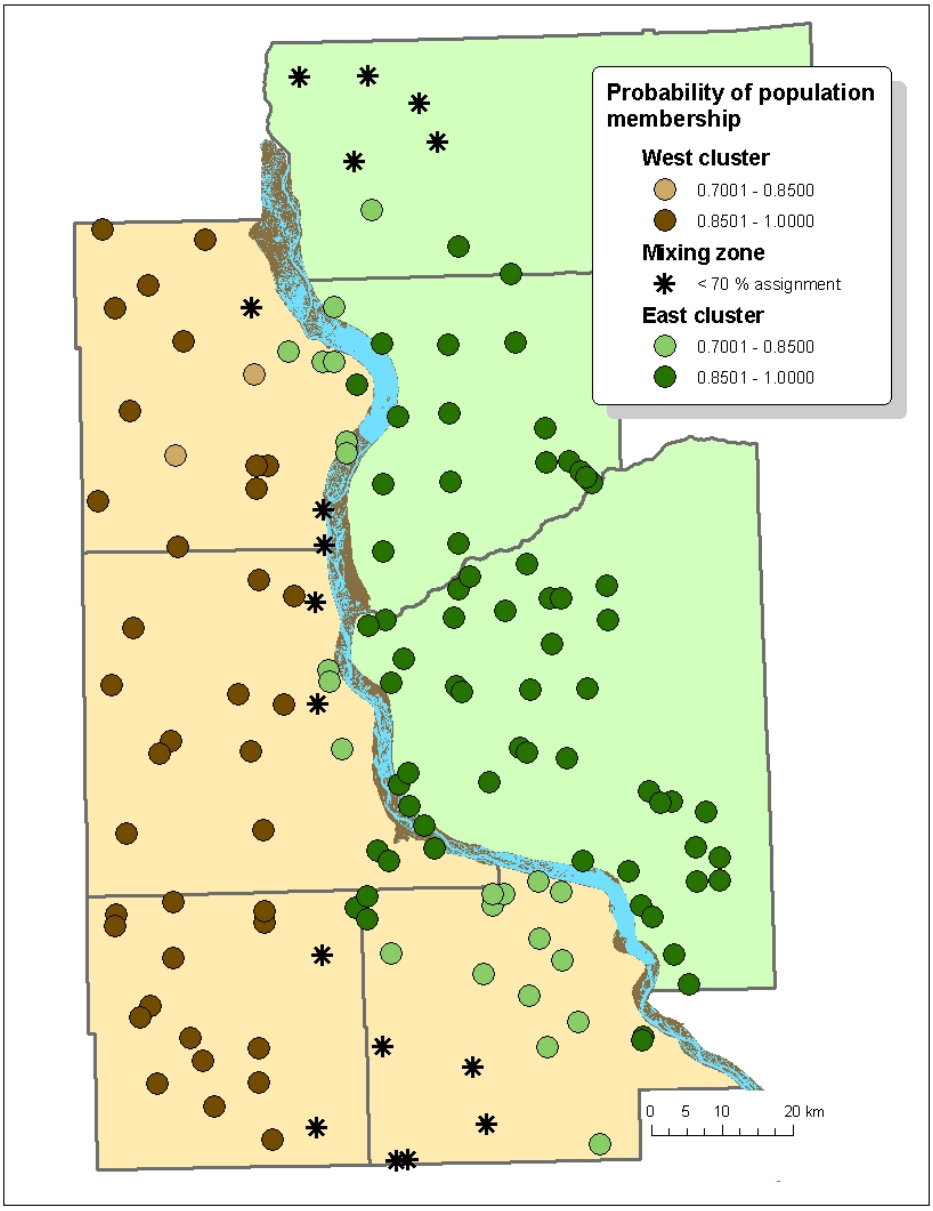

Figure 4. Mean probability of population membership for individual deer based on 20 runs of Geneland set at $K = 2$ using mtDNA data. Individuals belonging to the east (green dots) and west (brown dots) clusters identified are shown. We considered individual membership probabilities < 0.7 too low to be confidently assigned to a population, and instead considered them as belonging to a mixing zone (black stars).

Genetic differentiation: There were low but significant levels of genetic differentiation between Iowa and Wisconsin deer for microsatellites ($\Phi_{PT} = 0.005$, P = 0.003) and an order

of magnitude higher levels for mtDNA (Φ_{PT} = 0.052, P = 0.001). Pairwise measures of genetic differentiation between counties were likewise low for microsatellites, ranging from 0 to 0.019, with a mean of 0.009 for comparisons between pairs of counties on opposite sides of the Mississippi River and a mean of 0.002 for comparisons between pairs of counties on the same side of the river (Table 2a). Measures of genetic differentiation between pairs of counties were somewhat higher for mtDNA, ranging from 0 to 0.127, with a mean of 0.058 for comparisons between counties on opposite sides of the river and a mean of 0.044 for counties on the same side of the river (Table 2b). After the sequential Bonferroni correction, none of the pairwise Φ_{PT} values were significant for the microsatellite data, and only four were significant for the mtDNA data: Grant County (Wisconsin) versus each of the four Iowa counties (Table 2).

Isolation by distance: There was no significant evidence for a pattern of genetic isolation by distance at the spatial scale of a county. The microsatellite-based Mantel test of genetic differentiation (Φ_{PT}) against natural log-transformed Euclidean geographic distance among counties had a low correlation coefficient (r = -0.167) and was not significantly different from random (P = 0.294; Fig. 5a). The test using Cavalli-Sforza and Edward's chord distance produced similar results (r = -0.114, P = 0.380). For the mtDNA, the Mantel test of genetic differentiation (Φ_{PT}) against natural log-transformed Euclidean geographic distance also had very low correlation (r = -0.017) and did not show a significant difference from random (P = 0.475; Fig. 5b). Results from the test with Cavalli-Sforza and Edward's chord distance were likewise non-significant (r = 0.189, P = 0.227).

Table 2. Pairwise estimates of Φ_{PT} between counties (below diagonal) for microsatellites **(a)** and mtDNA **(b)** and significance values (above diagonal) based on 999 permutations in GenAlEx. Comparisons between counties separated by the Mississippi River are italicized.

(a)		**Iowa**				**Wisconsin**		
	County	Allamakee	Clayton	Delaware[1]	Dubuque	Crawford	Grant	Vernon[1]
Iowa	Allamakee		0.398	0.371	0.218	*0.008*	*0.100*	*0.071*
	Clayton	0.001		0.475	0.399	*0.010*	*0.013*	*0.150*
	Delaware[1]	0.005	0.000		0.439	*0.310*	*0.478*	*0.268*
	Dubuque	0.003	0.001	0.000		*0.014*	*0.488*	*0.471*
Wisconsin	Crawford	*0.011*	*0.012*	*0.009*	*0.011*		0.137	0.478
	Grant	*0.004*	*0.009*	*0.000*	*0.000*	0.004		0.218
	Vernon[1]	*0.019*	*0.013*	*0.019*	*0.000*	0.000	0.008	

(b)		**Iowa**				**Wisconsin**		
	County	Allamakee	Clayton	Delaware	Dubuque	Crawford	Grant	Vernon[1]
Iowa	Allamakee		0.413	0.136	0.016	*0.051*	*0.002**	*0.451*
	Clayton	0.000		0.116	0.010	*0.065*	*0.002**	*0.130*
	Delaware	0.020	0.019		0.185	*0.028*	*0.001**	*0.253*
	Dubuque	0.052	0.059	0.015		*0.012*	*0.001**	*0.175*
Wisconsin	Crawford	*0.039*	*0.031*	*0.051*	*0.060*		0.027	0.059
	Grant	*0.085*	*0.086*	*0.127*	*0.117*	0.043		0.024
	Vernon[1]	*0.000*	*0.043*	*0.028*	*0.033*	0.072	0.117	

[1] Counties with small sample sizes ($n \leq 8$). * Significant after sequential Bonferroni correction for multiple comparisons, starting at alpha = 0.0024.

There was a weak trend for a relationship between genetic and ecological distance, with the trend being stronger for mtDNA than for microsatellites. The microsatellite-based Mantel test of genetic differentiation (Φ_{PT}) against the ecological distance of crossing the river had a fairly high correlation coefficient ($r = 0.612$) with a positive slope that was not significantly different from random ($P = 0.122$). The test using Cavalli-Sforza and Edward's chord distance produced similar results ($r = 0.459$, $P = 0.104$). For the mtDNA, the Mantel

test of genetic differentiation (Φ_{PT}) against ecological distance also had a fairly high correlation (r = 0.627) with a positive slope that did show a marginially significant difference from random (P = 0.062). Results from the test with Cavalli-Sforza and Edward's chord distance were likewise weakly significant (r = 0.515, P = 0.065).

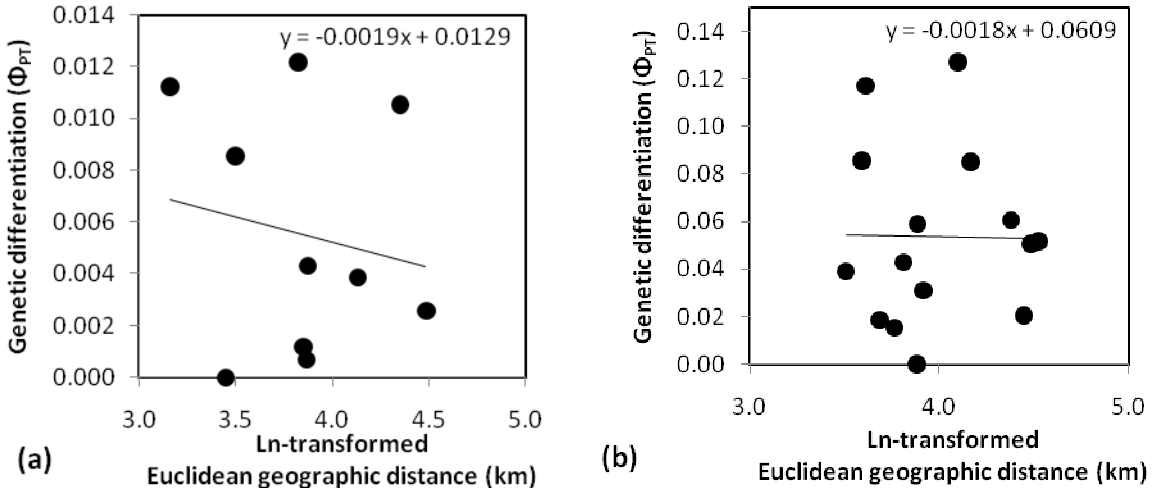

Figure 5. Mantel test of genetic differentiation (Φ_{PT}) calculated between counties using microsatellite data **(a)** and mitochondrial data **(b)**.

Significant spatial genetic structure in the form of individual-level genetic isolation by distance was found for both microsatellite and mtDNA data, but was much stronger for the mtDNA (Fig. 6). For microsatellite data there was weak but statistically significant genetic correlation (Moran's I = 0.0081; 95% CI around zero: -0.0060, 0.0066) at the first distance class (9.7 km). Although no other distance classes were significant, there was a general trend of decreasing genetic correlation with increasing distance size class and after the first five distance classes, the correlations remained consistently negative (Fig. 6a). For the mtDNA data there was considerably stronger correlation (Moran's I = 0.0698; 95% CI

around zero: -0.0267, 0.0333) at the first distance class, and correlations remained significantly positive through the first three distance classes, out to a distance of 29 km (18 mi). There was a general trend of decreasing genetic correlation with increasing distance, and for distances from 68 km to 87 km (42-54 mi) there was significant negative genetic correlation in the mtDNA data (Fig. 6b).

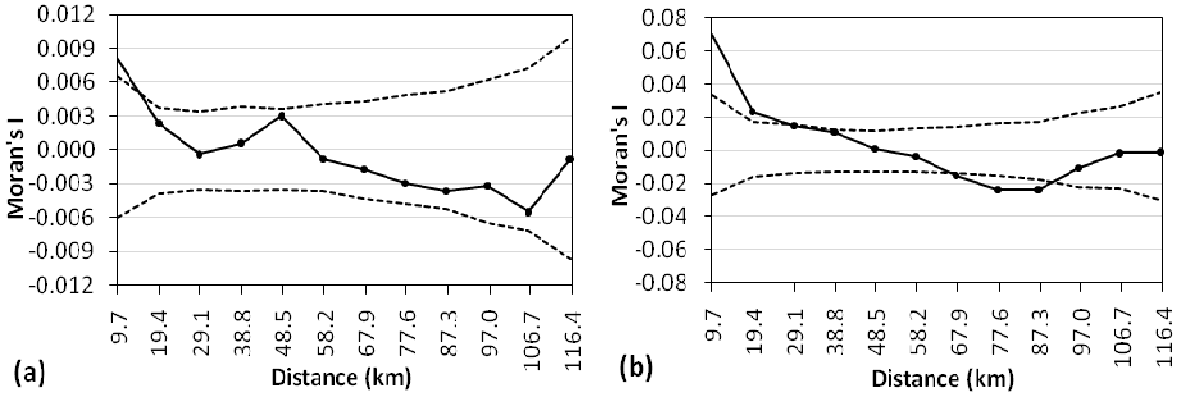

Figure 6. Plots of Moran's I calculated in SPAGeDi for microsatellites **(a)** and mtDNA **(b)**. All plots contain mean values (black circles) plotted at the end point of distance classes based on intervals of 9.7 km (6 mi) and include 95% confidence intervals (dashed lines) for the null hypothesis of no correlation based on 999 permutations.

Sex-biased dispersal: The estimated F_{ST} between Iowa and Wisconsin using the microsatellite data was 0.0026 (95% CI: 0.0006, 0.0046), which translated to an expected mtDNA F_{ST} of 0.0103 (95% CI: 0.0026, 0.0180) based solely on differences between the diploid biparentally-inherited microsatellites and haploid maternally-inherited mtDNA. The estimated F_{ST} from the observed mtDNA data was 0.0702 (95% CI: 0.0396, 0.1008), which is significantly larger than the expected mtDNA F_{ST}, which is consistent with there being less female gene flow between Iowa and Wisconsin than expected if both sexes dispersed equally

across the Mississippi River and providing indirect evidence of male-biased dispersal.

Discussion

We detected weak genetic structure in white-tailed deer across our relatively large study area in northeastern Iowa and southwestern Wisconsin (13,589 km^2) using several different analytical approaches. There was more genetic structure apparent with mitochondrial DNA than with microsatellites, as was expected given the evidence for male-biased dispersal in deer found in many other studies (Hawkins and Klimstra 1970, Purdue et al. 2000, Nixon et al. 2007) and differences in inheritance of the markers. We found no evidence for multiple discrete populations of deer in our study area using microsatellite data and limited evidence of two genetic clusters on either side of the Mississippi River when looking at data from mtDNA. Patterns of genetic isolation by distance were apparent at the spatial scale of the individual but not at the level of the county. There was weak evidence of increased genetic distance between counties separated by the Mississippi River relative to counties on the same side of the river for mtDNA data but not for microsatellite data. There was also evidence of sex-biased (specifically, male-biased) dispersal based on differences in genetic differentiation in our two different types of markers.

Based on our clustering analyses, it appears that deer in our study area represent one single population overall, with the two states connected primarily by male dispersers. Limited female dispersal across the Mississippi River is apparent in the mtDNA clustering results from Geneland that showed two genetic clusters. Although the boundary Geneland found between the two clusters of mtDNA did not correspond perfectly with the location of the Mississippi River, some earlier analyses in Geneland before the full data set was

completed (subset of n = 110) indicated two clusters with a boundary that *did* very closely follow the location of the river. Geneland has been shown to have trouble locating boundaries when there is greater than 30% connectivity between populations or when the contact zone between clusters is not a straight line (Chen et al. 2007). The movement of the cluster boundary depending on how many individuals were used seems to indicate that, while there was a signature of reduced permeability of the river to female dispersal relative to the rest of the landscape, the Mississippi River does not represent a hard boundary between discrete female populations but instead creates a zone of mixing in the area of the Mississippi River.

We did not find any pattern of genetic isolation by distance among deer when they were grouped at the level of a county, but there was a significant pattern at the individual level, indicating that what genetic structuring exists is at a fine spatial scale. Significant patterns of genetic isolation by distance at a spatial scale similar to that of our counties have been found in deer in South Carolina and Georgia using Mantel tests for both mtDNA haplotypes ($Z = 0.41$, $P = 0.039$) and biparentally-inherited allozymes ($Z = 0.70$, $P = 0.004$; (Purdue et al. 2000). However, the region studied by Purdue et al. (2000) is a coastal plains habitat, containing at least one site that was heavily (~95%) forested (Comer et al. 2005), and is very different from the row-crop agriculture-dominated landscape in which our study was conducted. The lack of county-level isolation by distance in our study area may be a function of the high rates and distances of deer dispersal, by both sexes, in agricultural landscapes that have been documented using telemetry (Nixon et al. 2007).

At the individual level, the pattern of isolation by distance observed was much stronger for spatial autocorrelation analysis of mtDNA data than for the microsatellite data, as might be expected based on the different inheritance modes of these markers and the

different dispersal ecology of the sexes, and suggests less dispersal by females than by males. However, although females apparently disperse less frequently than males, it does not mean that female dispersal does not occur. The significant spatial autocorrelation out to distances of 29 km for mtDNA indicates that females up to at least 29 km apart are, on average, genetically non-independent of each other (Diniz-Filho and Telles 2002). The mtDNA spatial autocorrelation we found extends out to a considerably greater geographic distance than that found in a study conducted in a semi-forested landscape of south-central Wisconsin, where spatial autocorrelation was significant out to only 6.4 km (Grear et al. 2010). This suggests that females in our study area are related at a larger spatial scale than in a less agricultural landscape. For microsatellites, our spatial autocorrelation out to 9.7 km was also greater than the 3.2 km distance Grear et al. (2010) observed and the 1.0 km distance observed by Comer et al. (2005) in a heavily forested landscape of South Carolina. This means that, even for a biparentally-inherited marker that is affected by dispersal of both sexes, there is genetic non-independence between females at a larger spatial scale in our study area than in others, possibly as a result of dispersal differences in different landscapes.

We found significant evidence, based on estimates of genetic differentiation from the two different types of genetic markers we used, that supported our hypothesis of male-biased dispersal across the Mississippi River. Male-biased dispersal has been demonstrated in other genetic studies of white-tailed deer, including one in South Carolina and Georgia where it was estimated from the comparison of mtDNA and biparentally-inherited allozymes that only 13% of total dispersal was by females (Purdue et al. 2000), and also in a study conducted in the southeastern United States where only 22% of total dispersal was by females (Ellsworth et al. 1994). Results of white-tailed deer telemetry studies in Illinois (Hawkins and Klimstra

1970, Nixon et al. 2007), Wisconsin (Skuldt et al. 2008), and Minnesota (Nelson and Mech 1984) also support male-biased dispersal, although the degree to which dispersal is male-biased may differ depending on the landscape because male and female dispersal rates differ in different habitats.

Our hypothesis that the Mississippi River is less permeable than terrestrial habitat to deer gene flow between Wisconsin and Iowa was supported by results from several of our analyses. Our Mantel tests of genetic distance versus ecological distance (Mississippi River) showed relatively high correlations ($r = 0.459 – 0.627$), and although the tests were not statistically significant at $P < 0.05$ for microsatellite data ($P = 0.104 – 0.122$), this may have been because of the small number of counties used in the tests. However, this pattern may still be a biologically significant one, such that the rate of deer dispersal between the two states is lower than within each state, and deer in Wisconsin may be less likely to disperse to the west across the Mississippi River than in another direction. For the mtDNA there was a stronger effect of the Mississippi River on genetic distance (P-values 0.062 to 0.065), indicating that the Mississippi River reduces gene flow of females, the traditionally philopatric sex, more than for males. We also found significant genetic differentiation between deer grouped by state for both microsatellites ($\Phi_{PT} = 0.005$, $P = 0.003$) and for mtDNA ($\Phi_{PT} = 0.052$, $P = 0.001$), with the difference again being stronger for females as indicated by the greater differentiation of the matrilineally-inherited mtDNA. Genetic differentiation between pairs of counties also weakly suggested some effect of the Mississippi River reducing deer dispersal since pairs of counties on opposite sides of this river tended to have higher values of Φ_{PT} on average than pairs on same side, for both types of markers. However, the level of genetic differentiation we observed is relatively weak, and

taken together with the weak effect of the Mississippi River in our Mantel tests, suggests that while the Mississippi River restricts gene flow somewhat, it is by no means impermeable to deer dispersal.

It is not surprising that the Mississippi River would have at least some effect on deer population genetic structure because rivers or lakes have been found to reduce gene flow and increase genetic differentiation in a number of other studies of fairly mobile mammal species. Sea lochs (long lakes) in Scotland affect red deer (*Cervus elaphus*) populations, resulting in significant population differentiation (microsatellite F_{ST} = 0.019; 99% confidence interval: 0.015 – 0.022) among populations separated by the lochs (Perez-Espona et al. 2008). In addition, the Niagara River between the United States and Canada has been found to genetically separate raccoon (*Procyon lotor*) populations, and also correspond to a break in the distribution of raccoon rabies, presumably as a result of the river restricting dispersal of infected individuals to Canada from the endemic areas in New York state (Cullingham et al. 2009). In Wisconsin, the rate of gene flow between white-tailed deer in a high CWD prevalence (core) area and other groups of deer in the area was found to be lowest for groups separated from the core by the Wisconsin River (Blanchong et al. 2008). The reduced gene flow across the river was correlated with lower CWD prevalence in these groups of deer separated from the high prevalence area by the Wisconsin River, indicating that the river probably reduced disease spread by dispersing infected individuals. The average microsatellite F_{ST} between sites on the north side of the Wisconsin River and the CWD core-area on the south side of the river was 0.0064 (Blanchong et al. 2008), which is greater genetic differentiation that we observed across the Mississippi River for our microsatellites F_{ST} = 0.0026 (95% CI: 0.0006, 0.0046). Given that CWD was found across the Wisconsin

River (although at lower prevalence levels) from the CWD core-area, and that the level of genetic differentiation is even lower across the Mississippi River, there appears to be considerable risk of CWD spread to Iowa across the Mississippi River by infected dispersing individuals. Since we observed evidence of male-biased dispersal across the Mississippi River, and male white-tailed deer have a higher prevalence of CWD infection than females (Grear et al. 2006), the risk of CWD spread is further compounded because the majority of the gene flow creating the low levels of genetic differentiation is by the sex that is most likely to be carrying the disease. It is important, however, to be aware that while dispersal of CWD-infected individuals may be the mechanism by which the disease could spread from Wisconsin to Iowa deer populations, other factors, such as local deer population density and habitat characteristics in Iowa, are likely to affect rates of disease establishment, transmission, and spread to other areas.

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CHAPTER 3. LANDSCAPE EFFECTS ON GENETIC STRUCTURE OF FEMALE WHITE-TAILED DEER IN AN AGRICULTURALLY DOMINATED MATRIX

A paper modified from a manuscript to be submitted to the Journal of Mammalogy

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Introduction

The landscape in which a species lives can affect dispersal and the degree of population genetic structure that results from those dispersal events, whether it is heterogeneity in harvested forests affecting American marten dispersal and spatial genetic structure (Broquet et al. 2006), existence of highway networks creating barriers that affect bighorn sheep dispersal and genetic diversity (Epps et al. 2005), or connectivity of woodland habitats affecting dispersal of European roe deer and genetic distances between individuals (Coulon et al. 2004). Since the population genetic structure of a species can be different depending on the characteristics of the landscape in which it is studied, studying genetic structure in a variety of landscapes is important. Results from studies in one landscape are not necessarily applicable to understanding ecology, disease spread, or conservation of the species in a different landscape.

The white-tailed deer (*Odocoileus virginianus*) is a good example of a species that is able to adapt and thrive in a variety of different landscapes. It is found across much of North America, in landscapes ranging from deciduous and coniferous forests to more open ranges

with broad plains or savannas (Hirth 1977). One aspect of the landscape that may influence deer dispersal is the amount of forest available for habitat. Forests and forest edges provide important cover for deer (Halls 1984) and reduce frequency of deer movements when compared to non-forested areas (Felix et al. 2007). The percentage forest cover was found to be highly correlated with both average and maximum dispersal distance ($R^2 = 0.94$ and $R^2 = 0.86$, respectively) in a meta-analysis of male white-tailed deer from various states across the U.S. (Long et al. 2005). Female deer may show a similar trend in dispersal being influenced by percentage of forest cover.

Aspects of deer ecology, especially dispersal and home range formation, shape spatial genetic structure of deer populations (Purdue et al. 2000, Comer et al. 2005). Specifically, the genetic similarity between deer is expected to be greater for individuals or groups that are spatially closer and to decline as the geographic distance between them increases, a relationship known as genetic isolation by distance (Comer et al. 2005, Blanchong et al. 2006). Limited female dispersal and the formation of adult home ranges near female relatives, where the home ranges of daughters may overlap and extend outward from the home range of their mother like the petals of a rose (Porter et al. 1991), results in matrilineal groups that are expected to be genetically related and aggregated in space (Mathews and Porter 1993, Aycrigg and Porter 1997, Nelson and Mech 1999). Landscape characteristics such as the amount of forest habitat may significantly impact deer ecology and thus spatial genetic structure of deer populations.

Although dispersal and population genetic structure might be expected to vary considerably in different landscapes, the majority of previous studies of female white-tailed deer population genetic structure have been limited to forested landscapes (Mathews and

Porter 1993, Comer et al. 2005) or landscapes such as coastal plains (Purdue et al. 2000) or plains and coastal islands of the southeastern United States (Ellsworth et al. 1994). There has been relatively little investigation of the characteristics of white-tailed deer population genetic structure in highly agricultural landscapes, although white-tailed deer certainly do occur in agricultural areas (Halls 1984, Nixon et al. 1991). In fact, deer flourish in intensive agricultural regions of the Midwest, and have generally benefited from the abundant source of food provided by crops, with corn and soybeans comprising a major portion of their diet (Halls 1984). However, the trend toward fewer and larger farms, with larger field sizes, limits the amount and diversity of forested habitat available to deer for cover, except along riparian systems and in more hilly areas (Halls 1984).

As result of limited forest habitat available in agricultural landscapes, deer dispersal dynamics, and resulting population genetic structure, may be different than in more forested landscapes. This is supported by recent telemetry work in heavily agricultural landscapes of Illinois with only 1.6 – 20% forest where female dispersal rates of 39 – 49% were found, and dispersal distances averaged 37 – 41 km (Nixon et al. 2007). By comparison, telemetry studies in landscapes with 54 – 97% forest that have found female dispersal rates of only 3 – 13% and average dispersal distances of only 4.5 – 8.0 km (Hawkins and Klimstra 1970, Comer et al. 2005, Skuldt et al. 2008). The potential differences in dispersal and population genetic structure in agricultural landscapes prompted us to study female deer population genetic structure in Iowa, where 67% of the land is in row-crop agriculture and only 9% is forested.

We took a landscape genetics approach to understanding white-tailed deer population genetic structure in Iowa. Landscape genetics, a combination of molecular population

genetics and landscape ecology, is the tool of choice for many modern-day studies of population genetic structure aimed at understanding the influence of landscape features on spatial patterns of population genetic structure (Manel et al. 2003, Sacks et al. 2004, Perez-Espona et al. 2008). While some other genetic approaches do incorporate a spatial component, landscape genetics adds an additional level of realism to analyses by explicitly incorporating information about features of the landscape in which individuals live, disperse, and interbreed. Landscape genetics is particularly useful for identifying landscape features that shape patterns of deer gene flow and population genetic structure in Iowa where there are no major landscape boundaries that could be expected to cause discrete populations.

Our overall objective was to characterize population genetic structure in female white-tailed deer in the agricultural landscape of northeastern Iowa where forest available for cover is very limited and fragmented. The specific objectives in this study were to: identify the number and location of populations and influence of landscape features on genetic connectivity between populations; assess whether there were patterns of genetic isolation by distance at two different spatial scales; and evaluate whether the amount of forest separating individual deer better explains the spatial pattern of population genetic structure than does simple geographic distance.

Study Area

The study area encompassed fifteen counties in northeastern Iowa (Fig. 1), an area of 23,802 km^2 with counties ranging in size from 1,139 km^2 to 2054 km^2. The area is characterized by extensive agricultural usage, with 27% of the land in corn, 12% in soybeans,

and 3% in other row crops, as well as 37% in pasture and hay. Forested area is very limited, at only 15% of the land, and what forest exists is extremely fragmented, except for some small continuous blocks of forest along riparian corridors and on bluffs near the Mississippi River.

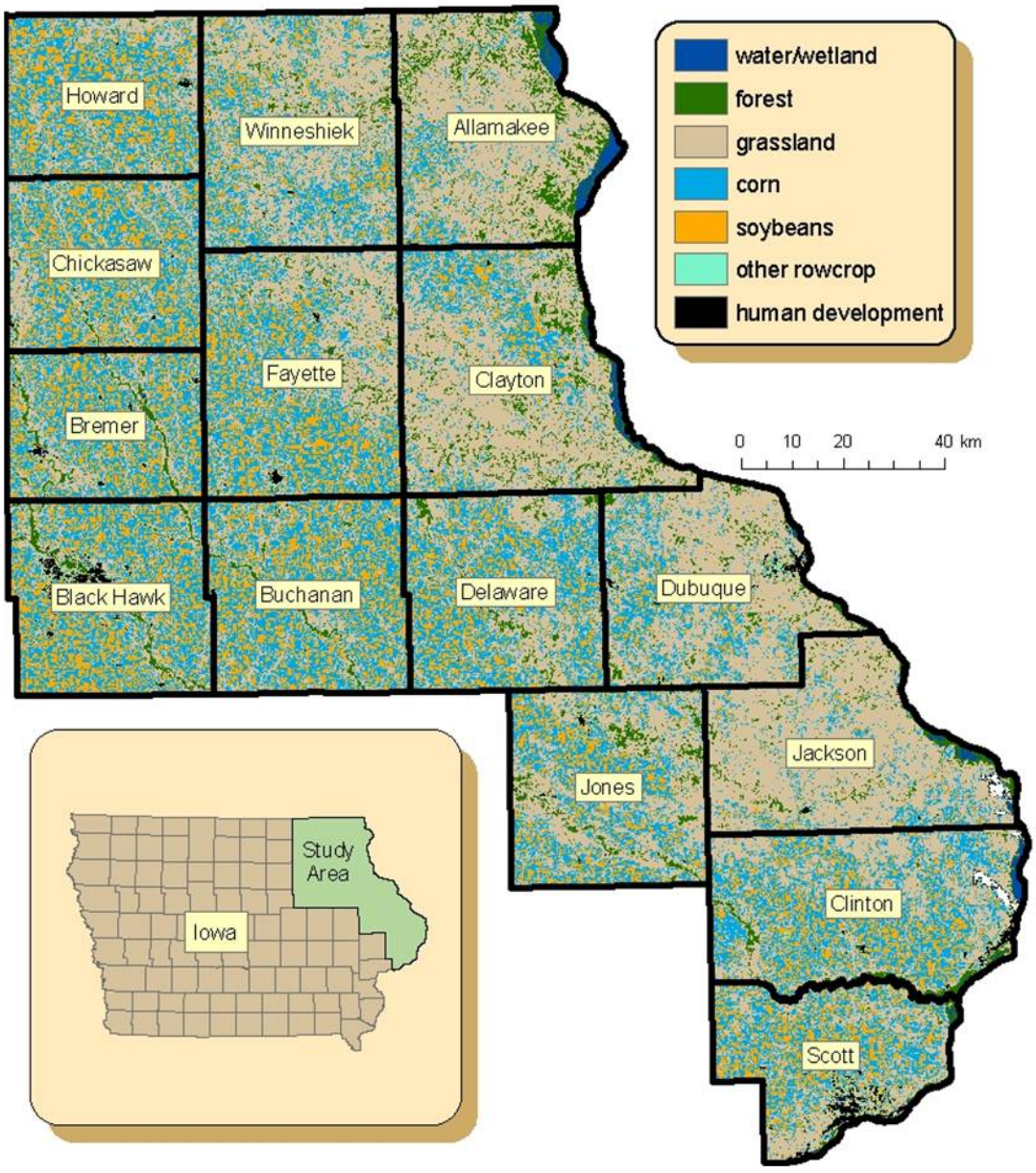

Figure 1. Major landcover categories in our study area in northeastern Iowa

Materials and Methods

Sampling:

We obtained samples of deer in 2006-2008 from the Iowa Department of Natural Resources (IDNR) that collected approximately 4,000 tissue samples (lymph nodes and/or brain stem) per year across Iowa (primarily in counties along the eastern edge of the state and somewhat sparsely in all other counties) to be used to test for chronic wasting disease (CWD). Most of the samples were from hunter-harvested deer during the fall hunting season, but a few samples were from road kill or deer that were targeted for CWD-sampling because of unusual behavior or exhibition of potential CWD symptoms. For the counties in our study area bordering the Mississippi River we used samples from 2006. In the counties further from the Mississippi River where sampling was much more limited, it was necessary to pool samples from all three years to achieve adequate sample sizes. During collection of tissue samples, spatial information on the location of harvest was collected by the IDNR. These data varied in spatial resolution from exact Universal Transverse Mercator (UTM) coordinates to sections, with the majority (93%) having section-level (2.6 km^2) spatial resolution.

From the available samples, a sample of female deer in each county was chosen, with as even geographic coverage as possible. We chose this sampling approach because there were no obvious landscape features that might create deer population boundaries by which we could predetermine the locations of populations from which to select samples. The even distribution of samples across the landscape was intended to give us the best basis for characterizing population genetic structure in the presumably continuously distributed

population across our study area, and is the most appropriate sampling scheme for use in clustering analyses aimed at identifying discrete populations (Schwartz and McKelvey 2009). Sampling design was constrained somewhat because the samples were collected in a somewhat opportunistic fashion by IDNR employees and do not represent a true random sample from each county. However, the available samples were approximately randomly scattered across the landscape in most of the counties.

Laboratory methodology:

We characterized population genetic structure in females using mitochondrial DNA (mtDNA), which is maternally inherited and therefore is not influenced by male dispersal movements. DNA for genetic analyses (n = 303) was extracted with a DNeasy® Blood & Tissue kit (Qiagen, Valencia, CA, USA). The tissue samples collected by IDNR included both the brainstem and retropharyngeal lymph nodes, but we used lymph tissue (whenever possible) because it appeared from our early lab work that lymph node tissue yielded higher concentrations and better quality of DNA than brain stem tissue.

We sequenced a 699 base-pair portion of the mtDNA control region (d-loop), a stretch of non-coding DNA, using PCR primers (forward: 5'-TCT CCC TAA GAC TCA AGG AAG -3', reverse: 5'- GTC ATT AGT CCA TCG AGA TGT C-3') developed by Miyamoto et al. (1990; Genbank Accession M35874). The section of mtDNA sequenced was the same as that sequenced in studies in Wisconsin (Grear et al. 2010). Primers were synthesized by the Iowa State University Office of Biotechnology's DNA Facility (hereafter, ISU DNA Facility). The control region was amplified by PCR in 12.5 µl volumes (2.5 µl DNA at a concentration of 20 ng/µl, 0.95 µl of 10X PCR buffer [Denville Scientific Inc., Metuchen, NJ, USA], 1.0 µl of

10 mM dNTPs, 1.0 µl each of 10 µM forward and reverse primers, 1.45 µl of 25 mM $MgCl_2$, 0.17 µl of Hot-Start Taq [Denville Scientific Inc., Metuchen, NJ, USA], 4.43 µl sterile DNA grade H_2O). Amplification conditions were: initial activation 95°C for 5 min, followed by 25 cycles with 94°C denaturation for 30 sec, 52.5°C anneal for 30 sec, and 68°C extension for 30 sec, with a final extension at 68°C for 3 min.

A small sample of PCR product was visualized on agarose gel to confirm successful amplification, after which a portion of the PCR product was purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) following the manufacturer's protocol to remove excess PCR reagents. The sequencing reaction was carried out at the ISU DNA Facility using primer and purified template provided by us. We used the reverse primer used in the amplification step as the sequencing primer, as we had difficulties with the nested sequencing primer developed by Miyamoto et al. (1990). This substitution of primers does not affect the section of the sequence we used for analyses or its comparability with studies in other states. Following the sequencing reaction, the sequence was run on the DNA Facility's ABI 3730xl DNA Analyzer.

The sequence data (traces) were visualized in Sequence Scanner v1.0 (Applied Biosystems, Foster City, CA, USA) and checked visually for correct base-calls, and trimmed to 581 bp to remove areas of sequence near the ends of the fragment that were of too poor quality for reliable base-calls. Sequences were then put into MEGA version 4 (Kumar et al. 2008) along with known haplotype sequences from previous work in Wisconsin (Grear et al. 2010) and aligned using the ClustalW algorithm (Thompson et al. 1994). The haplotype (representing all variable nucleotide sites leading to a unique sequence) of a sample was then determined by process of elimination when comparing it to all knowns. If a sample had a

unique, previously unobserved sequence that did not match any of the known haplotypes, we double-checked the sequence for correct base calls and then assigned it as a new haplotype. Quality control of the sequences was accomplished by including a blank sample (negative control) on each 96-well plate of samples submitted for sequencing. Samples that did not produce clean sequence on the first run were re-amplified and sequenced again. Correct assignment of sequences to haplotypes was checked independently by having a subset (10%) of samples assigned haplotypes by a second person and comparing these haplotypes to those assigned the first time.

Population genetic analyses:

Summary statistics: We calculated haplotype (*H*) and nucleotide diversity (π), as well as the total number of polymorphic sites and the number of transitions and transversions in Arlequin ver. 3.11 (Excoffier et al. 2005). We used a median-joining network (Bandelt et al. 1999) calculated in Network ver. 4.5.1.6 (http://www.fluxus-engineering.com/sharenet.htm; accessed 19 April 2010) and simplified by maximum parsimony algorithms (Polzin and Daneshmand 2003) to explore phylogenetic relationships among haplotypes. The median joining method finds minimum spanning trees (networks) made of the shortest connections between nodes (haplotypes here) and then uses maximum parsimony to delete connections that are not used by the shortest trees in the network, producing the simplest network connecting all nodes (Bandelt et al. 1999). We weighted transversions three times more strongly than transitions, as recommended in the Network user manual (Fluxus 2009) for mtDNA data. Only haplotypes present in the data set with a frequency greater than one were used in calculation of the network, both for clarity of the network and to reduce the

possibility of laboratory errors leading to false haplotypes that might influence the topology of the network. Haplotype nodes in the network were visualized as piecharts indicating the frequency of the haplotype in each county to explore whether individual haplotypes were spatially restricted in their distribution to certain regions of the study area or were found throughout the area.

Population clustering: We used both non-spatial and spatially-explicit Bayesian approaches for identifying the number of populations indicated by our mtDNA data. The non-spatial approach was conducted in Structure ver. 2.3.1 (Pritchard et al. 2000, Falush et al. 2003), a program that assumes a model with *K* populations and probabilistically assigns individuals to populations (or jointly to multiple populations if the admixture model is used and their genotypes indicated admixture) in such a way as to achieve Hardy-Weinberg equilibrium within populations (Pritchard et al. 2009). We implemented the admixture model, allowing the degree of admixture (α) to be different for each population, and set allele frequencies (λ) to be correlated between populations. Ten independent runs were conducted for each K = 1-5 using a Markov chain Monte Carlo (MCMC) approach with a burn-in period of 100,000 followed by 1,500,000 iterations. To determine the most likely number of populations, we plotted the log-likelihood values for each run against *K* following the graphical method of (Deyoung et al. 2009), where the best result was indicated by the highest value of the log-likelihood.

Our spatially-explicit Bayesian clustering analyses were conducted in the Geneland ver. 3.1.5 (Guillot et al. 2005b) package of the statistical program R ver. 2.10.0 (Ihaka and Gentleman 1996). Geneland uses spatial coordinate information in addition to the genetic

data to make inferences about the number of populations represented by the data set and the spatial location of boundaries between these populations. A MCMC technique is used to iteratively determine the most likely number of populations (*K*), with all values of *K* considered to be *a priori* equally likely, and cluster individuals into populations such that each population is approximately in Hardy-Weinberg and linkage equilibrium (Guillot et al. 2005a). We incorporated an uncertainty on the spatial coordinates of 1.6 km because most of the spatial data were collected at the level of a one-mile section (2.6 km^2). We ran the model five times allowing *K* to vary between minimum *K* = 1 and maximum *K* = 10. The maximum *K* was set to ten because we did not want to constrain iterations of the model to anything lower, although ten populations was much higher than what we expected might be reasonable for our study area. We conducted 500,000 MCMC iterations, with a thinning of 500, allele frequencies uncorrelated between populations, and all other values at their default settings. We used the uncorrelated allele frequency model because it is more robust than the correlated frequency model, even for data sets simulated with allele frequencies that are correlated (Guillot et al. 2005a). The most likely number of inferred populations was determined by looking at the modal *K* in a histogram of all *K* produced by the MCMC iterations and by counting the number of populations in the map output produced by Geneland.

Isolation by distance: In a large-bodied, mobile species, such as white-tailed deer, there may not be any sharp boundaries creating discrete clusters or populations even across a broad area of space as in our study. However, spatial genetic structure may occur at finer scales. Female philopatry and the establishment of adult home ranges in close proximity to other female relatives (Porter et al. 1991), may create a genetic gradient across the landscape with deer

that are in close proximity to each other being more genetically similar than those that are separated by greater geographic distances (i.e. isolation by distance). We tested for isolation by distance at 1) the level of deer management in Iowa (county) and 2) the individual level.

For the county-level tests of isolation by distance, we used Mantel tests (Mantel 1967), which performed a linear regression of a y-matrix of pairwise genetic distance between counties against an x-matrix of the corresponding pairwise geographic distances. The Mantel tests were performed in GenAlEx ver. 6.3 (Peakall and Smouse 2006) using 999 permutations to test for significance of the relationship. We used two measures of genetic distance. One measure of genetic distance was the degree of genetic differentiation between counties, this was calculated as Φ_{PT}, an analog of F_{ST} used for haploid data (Peakall and Smouse 2006), which we calculated in GenAlEx. The other genetic distance was Cavalli-Sforza and Edward's chord distance, that assumes genetic differences arise due to genetic drift and does not assume equal or constant population size (Cavalli-Sforza and Edwards 1967), and was calculated using the GenDist program of the Phylip ver. 3.69 suite of programs (Felsenstein 1989). The geographic distance between counties was calculated in the suite GeneticStudio build 131 (Dyer 2009) using the program Geno, Ln-transformed, and transferred to GenAlEx for use in the Mantel tests.

To test for patterns of isolation by distance at the individual level we used the genetic relationship coefficient Moran's I (Epperson and Li 1996), which is a measure of spatial autocorrelation used to detect departures from spatial randomness that indicate patterns such as geographic trends (Moran 1950). We used the program SPAGeDi ver. 1.3a (Hardy and Vekemans 2002) to calculate Moran's I and plotted it against binned inter-individual geographic distances in intervals of 9654 m (6 miles). The distance class size was a

compromise between narrower distance classes with fewer data points per class from which to calculate the estimate of Moran's I and wider distance classes where individuals separated by a wider range of distance would be lumped together and reduce our ability to detect fine-scale patterns. For all tests, 999 permutations were performed to produce a 95% confidence interval around the null hypothesis of no correlation between genetic and geographic distance.

Landcover effects on genetic structure: Given the strong influence that forest cover has been demonstrated to have on deer dispersal behavior (reviewed in the introduction), the amount of forest habitat between individuals may be a significant factor shaping patterns of spatial genetic structure in white-tailed deer. In order to investigate the effects of both geographic distance and forest cover (which are potentially correlated) on genetic distance, we used a partial Mantel test (Smouse et al. 1986). The partial Mantel test, an extension of a standard Mantel test, is a multiple regression where two or more pairwise distance matrices of predictor variables are combined with a matrix of a single response variable, and the partial correlation of the response with each predictor is calculated after taking into account the other predictor variable(s) (Smouse et al. 1986). Matrices of pairwise genetic and geographic distance between individuals were created in GenAlEx. We created an additional indicator matrix of pairwise "ecological" distance, as measured by the percentage of forest landcover along a straight line between two deer. We calculated the forest cover percentage in ArcGIS (ESRI, Redlands, CA) using the ET GeoWizards ver. 9.9 extension (ET Spatial Techniques, Faerie Glen, Pretoria, South Africa) to create a layer of lines between all pairs of individuals, which we then buffered at a distance of 30 m (the resolution of our landcover data) and

computed the percentage of forest within the linear buffer. Because calculating the forest cover for all pairs of individuals is extremely computationally intensive, we selected a random sample of 80 deer from the northern half of the study area to use for this analysis, an area representative of the range of landscape features throughout the whole study area. To conduct the partial Mantel test we used the program IBDWS (Jensen et al. 2005). We examined both the correlation between genetic and ecological distance when geographic distance was already accounted for ($r_{YXecol*Xgeog}$) and the correlation between genetic and geographic distance when ecological distance was already accounted for ($r_{YXgeog*Xecol}$). Standard Mantel tests of genetic against geographic distance (r_{YXgeog}) and genetic against ecological distance (r_{YXecol}) were also conducted for comparison. The correlations and partial correlations from the different tests were compared to identify which of the predictor variables best explained the pattern of genetic distance. We compared all the correlation coefficients (r) to identify whether adding a second predictor variable increased the fit of the model, i.e. increased r. If adding a specific predictor variable (such as forest cover) to the model substantially increased r then that predictor variable was considered to be adding to the ability of the model to explain the observed pattern of genetic variation.

Results

Summary statistics: A total of 81 polymorphic sites were identified in the 581 bp trimmed segment of sequenced mtDNA, resulting in 40 unique haplotypes. Of the 40 haplotypes observed, 11 were haplotypes previously identified in south-central Wisconsin by researchers at the University of Wisconsin – Madison (Grear et al. 2010) and were named using the same

alphabetical naming scheme (A, B, C, etc.). The other 29 haplotypes were new and we named them using the naming scheme ISU1, ISU2, ISU3, etc. Most of the polymorphic sites were transitions (n = 71), with only two transversions, sites where both a transition and a transversion occurred (n = 5), and sites with an insertion or deletion (n = 3). Haplotype diversity (*H*) was 0.924 ± 0.007 and nucleotide diversity (π) was 0.023 ± 0.011. In the median-joining network (Fig. 2) created using the 27 haplotypes that were observed > 1 time,

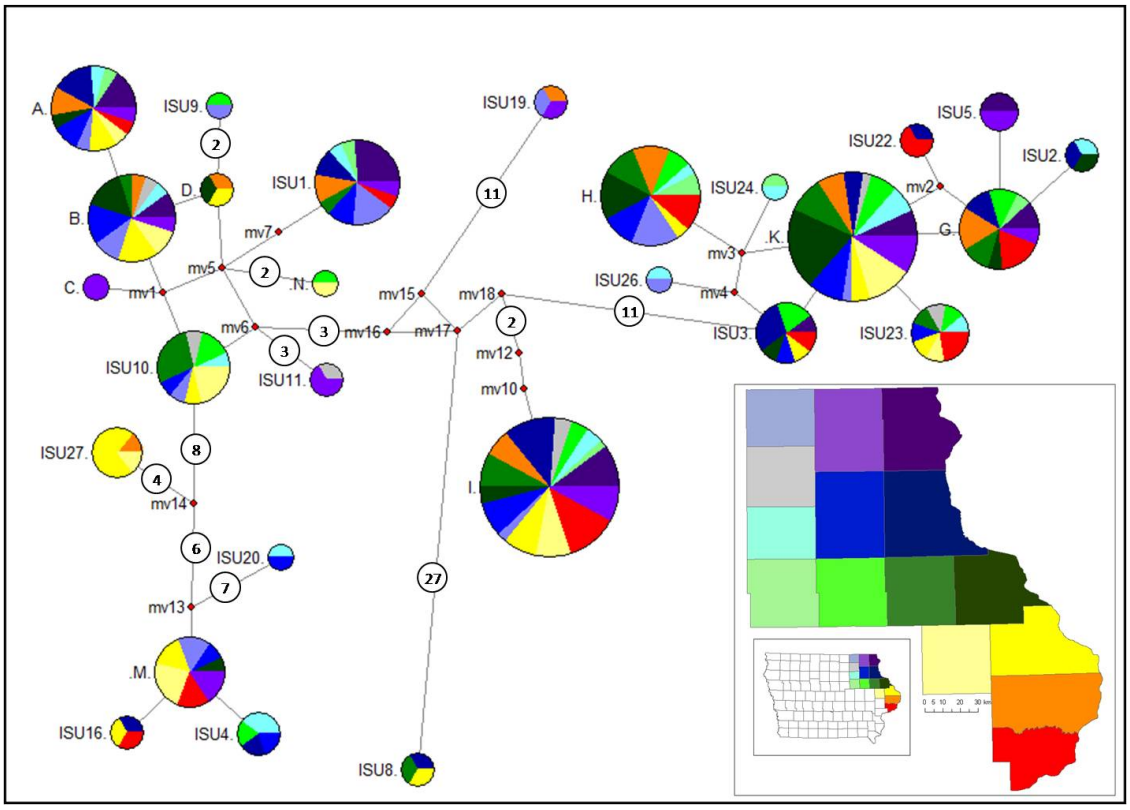

Figure 2. Median-joining network of 27 haplotypes post-processed by maximum parsimony calculated in Network. Haplotypes with frequency = 1 were not used (n = 13 haplotypes). Node sizes are proportional to the number of individuals possessing a haplotype, ranging from 2 – 50 individuals. Pie chart colors represent the 15 counties in the study area (see inset map). Alphabetically-named haplotypes (A, B, C, etc.) are those seen in previous work in Wisconsin (Grear et al. 2010) and alphanumeric names (ISU1, ISU2, ISU3, etc.) are those that are new to our study. Number of mutational steps between haplotypes are marked on branches for all branches ≥ 2 steps long.

many haplotypes connected to each other by only a single mutational step. However, some haplotypes were a large number of mutational steps different from any others. In addition, there were a few reticulations (cycles) where there was more than one possible parsimonious way in which the haplotype relationships could have arisen (Fig. 2). There was no observable pattern of spatial segregation of haplotypes across the study area, as indicated by the general lack of piecharts dominated by one or a few nearby counties (displayed in similar colors). The previously known haplotypes found by Grear et al. (2010) in south-central Wisconsin were dispersed throughout the network, with the new haplotype lineages we found interspersed between them.

Population clustering: In analyses using Structure, the highest estimated logarithm of probability of the data [Ln Pr(D|*K*)], averaged across ten runs at each value of *K*, occurred at $K = 1$ (Fig. 3), suggesting that deer in the study area comprise one continuous population. Some runs with $K > 1$ had much higher variability than runs with $K = 1$. The mean values of Ln Pr(D|*K*) did not follow an increasing trend with increasing *K* nor show any sign of reaching a plateau, so we did not apply the methods of Evanno et al. (2005) for using rate of change in Ln Pr(D|*K*) to identify the true number of genetic clusters.

Geneland indicated a single cluster as the modal number of populations, accounting for around 65% of the iterations (Fig. 4). Clustering with larger numbers of populations occurred fairly infrequently, accounting for fewer than 20% of iterations for any given value of *K*.

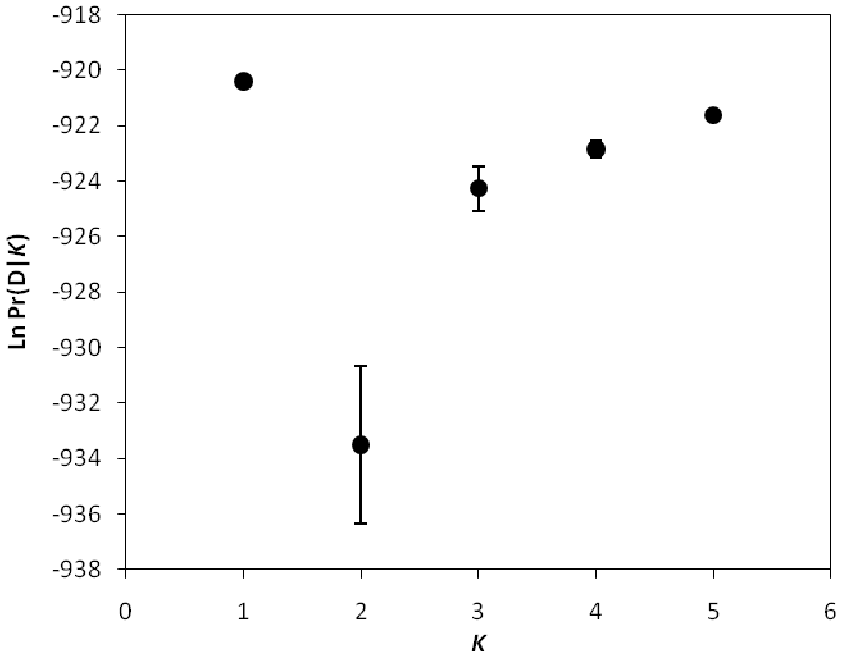

Figure 3. Mean and standard error of estimated log probability of the data [Ln Pr(*D*|*K*)] for each number of populations tested (*K*) in STRUCTURE over 10 independent runs at each value of *K*.

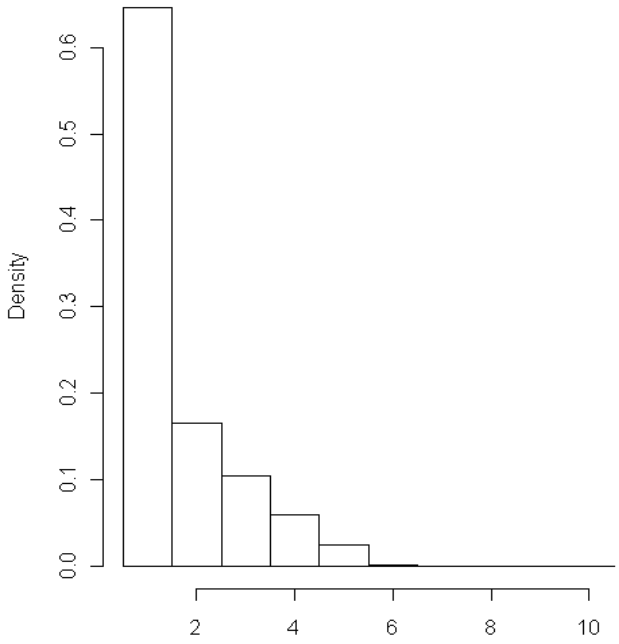

Figure 4. Number of populations along Markov Chain Monte Carlo chain summarized over 500,000 iterations (minus first 50,000 as burnin) run in Geneland.

Isolation by distance: The Mantel test of genetic distance (Φ_{PT}) against natural log-transformed Euclidean geographic distance had a very low correlation coefficient (r = 0.083) and a positive slope that was not significantly different from random (P = 0.244; Fig. 5a). The test using Cavalli-Sforza and Edward's chord distance also had a somewhat low correlation (r = 0.186) but had a positive slope that did show a significant difference from random (P = 0.031; Fig. 5b).

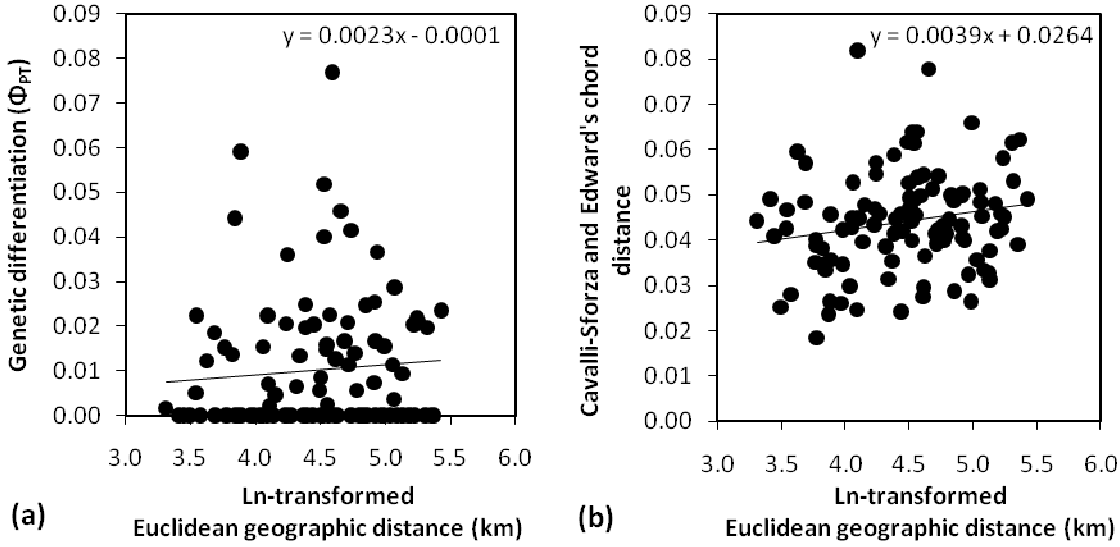

Figure 5. Mantel tests of genetic differentiation (Φ_{PT}; **a**) and Cavalli-Sforza and Edward's chord distance **(b)** calculated between all pairs of counties.

Significant spatial genetic structure in the form of individual-level genetic isolation by distance was found for deer within the first distance class (distance = 9.7 km, Moran's I = 0.0425; 95% CI around zero: -0.0195, 0.0224) and second distance class (distance = 19.4 km, Moran's I = 0.0154; 95% CI around zero: -0.0139, 0.0149; Fig. 6), indicating genetic non-independence between females separated by distances up to 19.4 km (12 miles). There was a

general trend of decreasing genetic correlation with increasing distance size class out through the first five distance classes, and later distance classes were somewhat variable but fluctuated near zero.

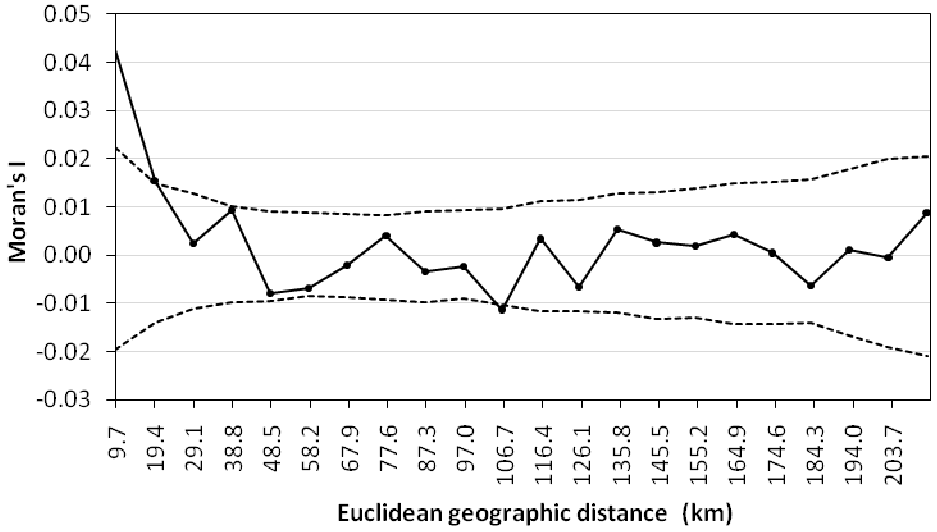

Figure 6. Plot of Moran's I calculated in SPAGeDi showing mean values (black circles) plotted at the end point of distance classes based on intervals of 9.7 km (6 mi) and include 95% confidence intervals (dashed lines) for the null hypothesis of no correlation based on 999 permutations.

Landcover effects on genetic structure: There was very low correlation in all of the partial and standard Mantel tests. The percentage of forest cover between pairs of deer was not significantly related to genetic distance between pairs of deer. The partial Mantel test of genetic against ecological distance when controlling for geographic distance ($r_{YXecol*Xgeog}$ = -0.0173, p = 0.27) explained the genetic data only slightly better than the standard Mantel of genetic against ecological distance (r_{YXecol} = -0.0171, p = 0.27). Likewise, the partial Mantel test of genetic against geographic distance when controlling for ecological distance ($r_{YXgeog*Xecol}$ = 0.0198, p = 0.15) explained the genetic data only slightly better than the

standard Mantel of genetic against geographic distance (r_{YXgeog} = 0.0196, p = 0.15). The percentage forest cover between pairs of individuals was heavily skewed to the left (Fig. 7), as there were few if any locations in our agriculturally dominated study area where individuals were connected by land with high percentages of forest.

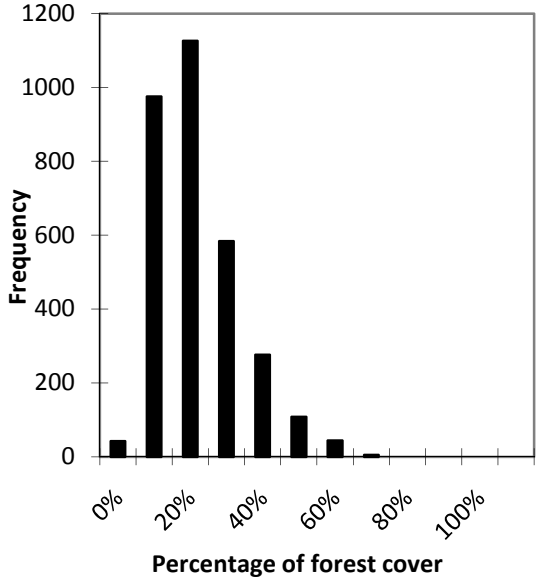

Figure 7. Histogram of percentage forest cover between pairs of individuals.

Discussion

Unlike what has been demonstrated many times in deer populations in forested habitats, we detected only weak genetic structure among female white-tailed deer in our agriculturally-dominated study area in northeastern Iowa. There was no evidence of multiple unique genetic clusters across our relatively large study area (23,802 km^2), but there was a weak pattern of genetic isolation by distance at both the level of the county and at the individual level. Although forests have been shown to be important to deer for providing cover (Hirth 1977, Halls 1984, Felix et al. 2007), the absolute percentage of forest cover

between individual females did not explain patterns of genetic distance in our study area.

Based on our clustering analyses, it appears that female deer in northeastern Iowa represent one single population without any discrete boundaries. This suggests that there are fairly high levels of gene flow throughout the region, even among females, a conclusion that is supported by the distribution of the haplotypes we observed and the configuration of the haplotype network. There were few, if any, haplotypes that were restricted to one region of the study area, and many of the haplotypes that we observed at high frequencies were each found in a large number of counties. This is in stark contrast to the results of Purdue et al. (2000) who looked at mtDNA haplotypes in South Carolina and Georgia and found that sites only 50 km apart rarely had any haplotypes in common, indicating high levels of female philopatry in their study area. The configuration of the haplotype network also suggested high levels of gene flow not only within our study area, but across larger areas as well. Haplotypes that had previously been observed in deer from south-central Wisconsin (Grear et al. 2010) were observed in our study area, over 100 km away, and many of the new haplotypes we observed were closely related to the haplotypes from Wisconsin. However, our haplotype frequencies, with our most common haplotype at 16.5%, were very different from those observed in south-central Wisconsin where 78% of deer shared a single common haplotype, although this may be a function of our study being at a much larger scale the 310-km^2 area studied by Grear et al. (2010).

The distribution of haplotypes we observed may also have been affected by the history of past deer population declines, reintroductions, and population rebounds in the area. Although white-tailed deer were abundant in the Midwest before the time of European settlers, populations experienced serious declines by the 1880s to 1890s as a result of market

hunting (Halls 1984) and severe winters in 1848, 1856, and 1880 (Stone 2003). In Iowa, deer were virtually wiped out, causing all hunting to be banned in the state in 1898, and despite restoration efforts by state officials and a few private citizens with captive herds, deer had only recovered to an estimated 500 – 700 deer in the entire state in 1936 (Stone 2003). Increases in deer populations in northeastern Iowa were aided partly by deer moving back into the state on their own and also by deer from captive herds (containing some deer originally from Nebraska and Minnesota) that were transplanted into the northwestern corner of Dubuque County in our study area in 1943 (Stone 2003). The reintroduction of deer that may have had mtDNA haplotypes from matrilines in Nebraska and Minnesota (or elsewhere), plus the movement of deer back into our study area from outside the state, make it difficult to determine which haplotypes might have come from outside the state and which might be from matrilines native to northeastern Iowa. For example, we observed two haplotypes (ISU19 and ISU8) which were a large number of mutational steps different (at least 11 and 27 steps, respectively) from any other haplotypes and could potentially represent out-of-state matrilines of deer from the reintroduction event. A continent-scale assessment of where different haplotypes are found would help to determine which, if any, of the haplotypes we observed might have come from the reintroduced deer from other states.

Despite the large number of unique haplotypes identified in the study area and their lack of clear spatial clustering, we did find evidence of isolation by distance at both the county and individual levels. However, the pattern at the county level was fairly weak, as indicated by the low correlation for the Mantel test with Cavalli-Sforza and Edward's chord distance ($r = 0.186$, $P = 0.031$) and the lack of significant pattern in the Mantel test with Φ_{PT} ($r = 0.083$, $P = 0.244$). A significant pattern of genetic isolation by distance at a spatial scale

similar to that of our counties has also been found in deer in a coastal plains habitat of South Carolina and Georgia (Purdue et al. 2000). At the individual level, we observed significant spatial autocorrelation out to 19.4 km (12 miles), indicating that female white-tailed deer within 19.4 km are genetically non-independent (Diniz-Filho and Telles 2002). The spatial autocorrelation extends out to a considerably greater geographic distance than that found in a study conducted in a semi-forested landscape of south-central Wisconsin using the same region of mtDNA where spatial autocorrelation among deer was significant only out to 6.4 km (Grear et al. 2010). One possible explanation for why we found significant spatial autocorrelation out to a distance more than three times the distance found by Grear et al. (2010) is that female dispersal rates and distances in our agriculturally-dominated landscape are greater than those of females in the more forested landscape of south-central Wisconsin. This would lead to closely related females being farther apart in our landscape than in Wisconsin and create population genetic structure at a larger spatial scale. Greater female dispersal rates and distances in an agricultural landscape were documented in a telemetry study by Nixon et al. (2007) in Illinois with only 1.6 – 20% forest that found 22 – 49% dispersal rates and 37 – 41 km dispersal distances. This contrasts with a telemetry study conducted in a roughly 50% forested landscape in south-central Wisconsin where only one out of 32 (3%) females dispersed (Skuldt et al. 2008).

Despite the importance of forest cover to white-tailed deer (Halls 1984) and the finding that percentage of forest cover is highly correlated with dispersal distance in male deer (Long et al. 2005), we did not find that percentage of forest cover described patterns of genetic distance on the landscape. Although the correlation of forest cover with genetic distance was not significant, it is interesting to note that the correlation did have a negative

sign, meaning that deer separated by more forested areas were more genetically similar than deer separated by less forest. This pattern, although non-significant, suggests that there is perhaps some trend of forest connecting female deer, potentially via dispersal through forest. There are a number of possible reasons why we may have failed to detect a relationship between genetic distance and percent forest cover if there is one. It may be that there is too much variability in the relationship between genetic and forest cover data at the individual level to find a pattern that does exist. Alternatively, it may be that because most of the deer were separated by only a small percentage of forest cover (see Fig. 7), there was not a wide enough range of forest cover to detect a significant effect on genetic structure. We may have been able to detect a stronger pattern if we had deer separated by the full range (0 – 100%) of possible of forest cover percentages. Finally, it is possible that some other aspect of forest cover, such as its configuration or its degree of fragmentation, influences dispersal and genetic structure more than simply the overall percentage of forest separating animals.

Despite what has traditionally been observed in other studies, female white-tailed deer in northeastern Iowa exhibited very weak population genetic structuring. One potential explanation for our results is that a high percentage of agriculture and limited forest may increase dispersal rates and/or distances in female deer even though dispersal has traditionally been thought to be rare for females. This is consistent with findings from a recent telemetry study that documented high rates of female dispersal in an agricultural region in Illinois (Nixon et al. 2007). The increased gene flow resulting from high rates of female dispersal would result in increased genetic homogenization across the landscape and less detectable population genetic structure. Another potential explanation for weak female genetic structure in northeastern Iowa is the relatively intense harvest in this region, with

47% of deer in each county harvested annually (IDNR 2009), of which 44 – 60% were females in the counties included in our study area (Litchfield 2008). It has been suggested that intense harvest, and the young age structure that it creates, may reduce the degree of genetic structure in an area by reducing the number of related females with home ranges near each other (Comer et al. 2005). In deer populations with no hunting pressure, females have been found to develop relatively high levels of spatial genetic structure as a result of related females establishing home ranges in close proximity to each other (Mathews and Porter 1993). A third possible explanation for the observed weak genetic structure in northeastern Iowa is that deer harvest activities may disrupt home ranges and social structure. In Iowa, a common strategy of hunting is 'driving', where a few hunters are stationed in open agricultural fields and several other hunting companions drive the deer out of wooded patches toward the stationary hunters (Stone 2003). The disturbance created by the presence of hunters has been found to result in changes to deer behavior, such as increased movement in order to seek refuge from hunters and increases in home range size as a result of these hunter avoidance behaviors, especially relative to less disruptive deer harvest methods such as sharpshooting (Williams et al. 2008). Because the spatial location data for our deer is the location of harvest, and a deer may have enlarged its home range in an effort to avoid hunters or been harvested outside of its home range due to movement cause by deer driving, our spatial information may not represent the true location of the deer's established home range. This uncertainty in the true spatial location of the observed genetic information might hinder our ability to detect spatial genetic structure, although the spatial scale of most of our analyses was much greater than the spatial uncertainty that would be created by deer harvest. The above explanations for the weak female population genetic structure we observed in

northeastern Iowa are not mutually exclusive, so the true reason may be a combination of these explanations.

The weak female genetic structure observed in our agriculturally-dominated landscape, suggesting high mobility of deer across the landscape, has important ramifications for predicting disease spread because higher mobility increases the potential for disease to spread across an area via dispersing, infected individuals. Weak genetic structure in agricultural landscapes also has implications for identifying the appropriate scale for management of deer populations, which may be a different scale than would be appropriate for deer populations exhibiting higher levels of spatial genetic structure.

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CHAPTER 4. GENERAL CONCLUSIONS

General Findings

In this project, I characterized the degree of genetic connectivity between deer populations in Iowa and Wisconsin to identify factors influencing the risk of chronic wasting disease (CWD) entering Iowa from Wisconsin via the natural movement of free-ranging white-tailed deer (Chapter 2) and characterized the population genetic structure of white-tailed deer in northeastern Iowa to understand effects of landscape features on female spatial genetic structure (Chapter 3). General findings from these chapters are briefly reiterated below for the purpose of refreshing the reader's memory before the discussion of possible applications of my findings to management of deer in Iowa. I refer the reader to the discussion sections of Chapters 2 and 3 if additional details or comparisons of my results with other studies are desired.

General findings from Chapter 2 are as follows: Clustering analyses indicated that deer in my study area represent one population overall, with Iowa and Wisconsin connected primarily by male dispersal. I did not find any significant pattern of genetic isolation by distance at the level of a county (the current unit of deer management in Iowa), but there was a significant pattern at the individual level, indicating that what genetic structure exists is primarily at a finer spatial scale than the county. I found indirect evidence of significantly higher male than female dispersal across the Mississippi River. I also determined that the Mississippi River is quite permeable to gene flow (dispersal) and has only a weak effect on population genetic structure of deer in my study area.

General findings from Chapter 3 are as follows: Based on mtDNA data, my clustering analyses indicated that female deer in northeastern Iowa represent one population without any discrete boundaries. However, I did find evidence of genetic isolation by distance at both the county and individual levels, indicating that although female deer in northeast Iowa are one population, there is still some population genetic structure in the region. However, I found that the patterns of spatial genetic structure observed did not significantly correlate with the absolute percentage of forest cover (preferred deer habitat) separating individuals.

Implications for Management

As mentioned in Chapter 1, information regarding white-tailed deer population genetic structure within my study area could be used as a basis for fine-tuning the IDNR Wildlife and Law Enforcement Bureau's Chronic Wasting Disease Response Plan (hereafter, The Plan; (IDNR 2009), both in terms of informing recommendations for sample sizes and locations for yearly surveillance of CWD and for suggesting changes to sizes of surveillance and depopulation zones should CWD be detected in Iowa.

First I address how my results might be used to fine-tune yearly CWD surveillance sampling. One of the original goals that prompted my study was the identification of specific landscape features, such as stretches of the river with a narrow channel and high density of islands rather than wide pools, affecting dispersal of deer across the Mississippi River. I had hoped that if specific landscape features of the Mississippi River were found to correlate with locations of increased genetic connectivity, the information could be used to assist the IDNR in fine-tuning its CWD sampling locations along the Mississippi River to have the best

chance for the early detection of CWD in Iowa resulting from the movement of infected animals from Wisconsin. Because of the relatively weak population genetic structure of white-tailed deer in northeastern Iowa and southwestern Wisconsin, I was unable test the correlation of fine-scale landscape features of the Mississippi River with patterns of genetic structure.

However, although there was not sufficient genetic structure to test specific features of the Mississippi River, results of my tests of population genetic structure in my study area could still be used to help the IDNR refine CWD sampling efforts. My findings of the apparently high permeability of the Mississippi River to gene flow and thus potential for CWD spread via infected dispersers crossing the river, underscore the importance of the CWD surveillance conducted by the IDNR. According to The Plan, recommendations for the 2009-2010 hunting season are to sample 500 deer per county in the five counties (Allamakee, Clayton, Delaware, Dubuque, and Jackson) in northeast Iowa along the Mississippi River, with an additional 500 deer from Clinton and Scott counties combined, plus 1000 roadkill samples statewide. The weakly significant genetic isolation by distance that I found at the county level in northeastern Iowa (Chapter 3) and individual-level isolation by distance in the form of spatial autocorrelation that I observed in females up to 29 km (18 miles) apart suggest that female deer are capable of moving considerable distances within my study area. The indirect evidence of male-biased dispersal I observed (Chapter 2) would lead to even less genetic structure in males than I observed in females and suggests even greater movement by males. Therefore, the IDNR may wish to consider expanding intensive CWD sampling, such as is currently done in the five northeast Iowa counties along the Mississippi River, to more than one county-width away (to the west) from the Mississippi River, especially because

CWD prevalence can exceed 1% before clinical cases are first detected in an area (Miller et al. 2000). How much further out from the river this would be than current sampling would depend on available resources of money, time, and personnel.

My results also provide information that could be used as a basis for fine-tuning the radius of zones of increased surveillance and depopulation if a CWD-positive deer is detected in Iowa. The Plan currently proposes a five-mile (8.0 km) radius surveillance zone of increased sampling around the location of any CWD-positive case (either a free-ranging deer or an individual from a captive cervid facility), and should additional cases be detected from the increased surveillance, a five-mile radius zone where depopulation of all free-ranging cervids is planned in an effort to eradicate the disease. The currently proposed localized depopulation is reminiscent of a management strategy proposed by Porter et al. (1991) in New York for controlling deer population sizes in localized areas where extreme population reductions or total depopulations were desired. The study by Porter et al. (1991), conducted in the Adirondack Mountains suggested localized deer depopulation could be achieved as a result of the social structure exhibited by female deer. Their study found that female deer were highly philopatric and established adult home ranges adjacent to and overlapping with those of their mother and other female relatives, much like the overlapping petals of a rose, hence this is known as the “rose-petal hypothesis”. The authors suggested that localized depopulation could be achieved by removing all females from a certain small location and that recolonization by other deer would be extremely slow (taking five to ten years) because of a lack of female dispersers due to very strong female philopatry, high levels of female fidelity to established home ranges in the surrounding area, and no reason for males to colonize areas where mates were lacking.

If deer in Iowa exhibited population structuring similar to the deer in the Adirondack Mountains where the localized depopulation management strategy of Porter et al. (1991) was developed, then the five-mile radius depopulation zones suggested by The Plan could possibly be adequate to restrict spread of CWD upon detection in Iowa. Although, for the localized depopulation to be effective at restricting CWD spread, it would be necessary to remove all deer (as is currently stated in The Plan), not just females as in the method of Porter et al. (1991), and depopulation would need to be maintained for several years to reduce the risk of environmental transmission from infectious prions remaining in the environment (Miller et al. 2004, Mathiason et al. 2009). However, I do not believe that localized depopulation in a five-mile radius zone would be adequate to achieve the IDNR goal of CWD eradication in Iowa if it is detected in the state. Based on the weak levels of genetic structure that I detected in my study area and the existence of significant spatial autocorrelation between females as much as 29 km (18 miles) apart, both of which suggest relatively higher rates and distances of female dispersal than commonly documented in more forested habitats, the management approach suggested by Porter et al. (1991) would probably not be appropriate for Iowa because the depopulated zone would likely be recolonized much more rapidly than the 5-10 years that they estimated. The existence of significant spatial autocorrelation between females up to 18 miles apart, and evidence of male dispersal being even greater than that exhibited by females, suggests that a much larger radius than five miles may be needed for CWD surveillance and depopulation zones. The need for larger depopulation zones is also supported by the fact that genetic data provides evidence of only the dispersal events that result in successful breeding and contribution of genes to the destination population (Slatkin 1994) and does not account for animals that disperse and do

not breed or animals that make exploratory movements. This results in genetics giving only a minimum estimate of the true amount of deer movement across the landscape. Deer movement has been found to be very high in agriculturally-dominated regions of Illinois with only 1.6 – 20% forest, where 65% of males and 39% of females dispersed, and average dispersal distances of 28 – 44 km for males and 37 – 41 km for females were observed (Nixon et al. 2007). If dispersal distances and rates in northeastern Iowa, where the landscape is also agriculturally-dominated and has only 15% (highly fragmented) forest habitat, are similar to those observed by Nixon et al. (2007) in Illinois, then depopulation zones with a radius larger than five miles (8.0 km) could be particularly important for restricting CWD spread if it is detected in Iowa.

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APPENDIX. ESTIMATION OF NUMBER OF DISPERSERS ACROSS THE MISSISSIPPI RIVER

We present here a rough estimation of the number of deer dispersers per generation across the Mississippi River. These estimates should be used with caution because of the caveats (see below) associated with this relatively simplistic approach.

Based on our calculations in Chapter 2, the estimated genetic differentiation between deer in Iowa and deer in Wisconsin for biparentally-inherited microsatellites that are affected by dispersal of both sexes was F_{ST} = 0.0026 (95% confidence interval: 0.0006, 0.0046), and for maternally-inherited mitochondrial DNA (mtDNA), which is affected only by female dispersal, genetic differentiation was F_{ST} = 0.0702 (95% confidence interval: 0.0396, 0.1008). These estimates of genetic differentiation (F_{ST}) between Iowa and Wisconsin can be translated into estimates of the number of dispersers per generation (Nm) that cross the Mississippi River using the equations of Slatkin (1994), which are $Nm = [(1/F_{ST}) - 1]/4$ for microsatellites, and $Nm = [(1/F_{ST}) - 1]/2$ for mtDNA. The results of the conversion are an estimated 96 deer (95% confidence interval: 55 – 390 deer) crossing the river each generation and contributing their genes to the gene pool on the other side. Of the dispersers, an estimated 7 are females (95% confidence interval: 4 – 12 females). This means 7.3% of dispersers across the river are female, which is fairly comparable with results from a study in the southeastern United States that found 13% of dispersers were female in an area of coastal plains with the Savannah River running through it (Purdue et al. 2000).

There are several important reasons why estimates of dispersal produced by this method of estimating Nm from F_{ST} should be interpreted cautiously. Slatkin (1994) and

Whitlock and McCauley (1999) put forth several of these reasons, which include:

1) Estimates of dispersal from genetic methods represent a historical picture from many years of dispersal events averaged over the entire set of subpopulations and are not sensitive to recent changes in gene flow such as those caused by recent changes to the landscape through which individuals disperse.

2) Translating F_{ST} is an indirect method of estimating *Nm*, and therefore depends on the assumptions made about processes affecting allele frequencies within populations and genetic differentiation among populations, especially the assumption that the populations in question are at genetic and demographic equilibrium. Another important assumption is that the migration rate is much larger than the mutation rate. But as long as this assumption is met, which is generally reasonable biologically, the mutation rate has relatively little effect on F_{ST}. Another assumption is that there is no selection affecting the markers, which should not be a problem for our data because all our markers are non-coding regions and presumably neutral to selection. Other assumptions include that there are an infinite number of equal-sized populations exchanging dispersers and that all populations are equally likely to exchange dispersers regardless of their geographic location.

3) Detecting evidence of dispersal via its effect on genetic differentiation requires not only that the individual be involved in a dispersal event, but also that the individual is either sampled itself or breeds and leaves its genetic signature in the population and one of its descendants is sampled. As a result, any gene flow detected by genetic differentiation is a minimum measure of the true amount of dispersal.

Another problem with estimating gene flow (*Nm*) from genetic differentiation (F_{ST}), especially for the low levels of F_{ST} found in our study, is that slight changes in the lower (less

than about 0.01) values of F_{ST} can result in large changes in the estimated value of *Nm*, which we demonstrate with a simple plot of Slatkin's (1994) equations (Fig. A1) of a number F_{ST} values translated to *Nm*. Thus, the estimates of *Nm* produced from low values of F_{ST} are extremely imprecise.

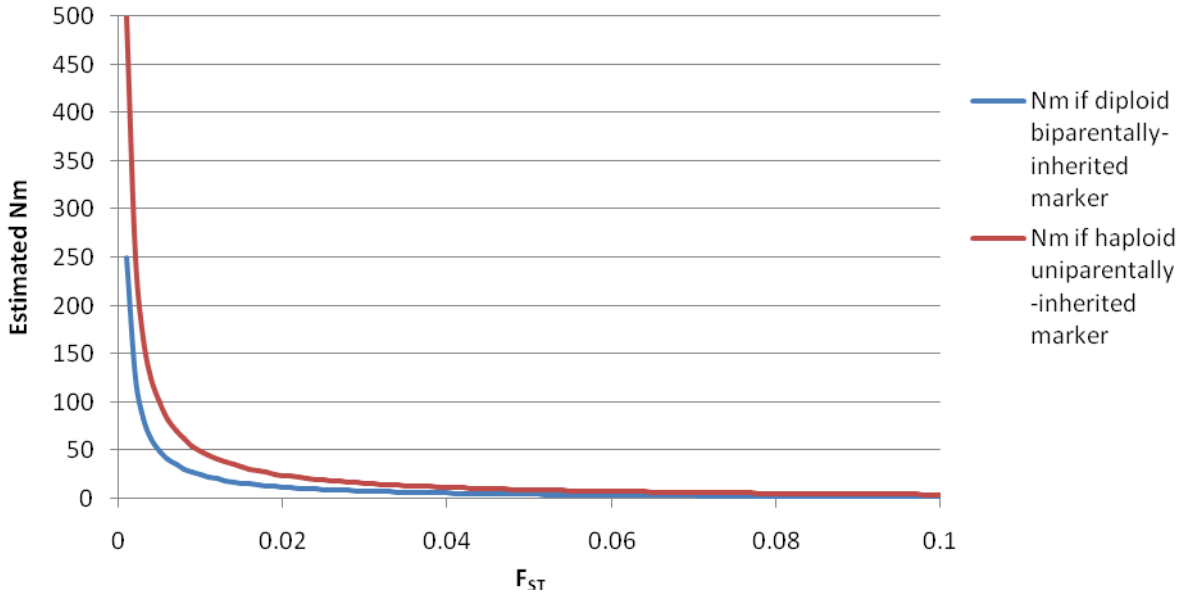

Figure A1. Estimated number of dispersers per generation (Nm) based on degree of genetic differentiation (F_{ST}).

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