WHITE-TAILED DEER POPULATION STRUCTURE IN GEORGIA: CONSEQUENCES OF RESTOCKING AND POTENTIAL INFLUENCES ON THE DISTRIBUTION OF HEMORRHAGIC DISEASE MORTALITY

By

SABRINA NICOLE MCGRAW

(Under the direction of Elizabeth Howerth)

ABSTRACT

Hemorrhagic disease (HD) is the most significant infectious disease of white-tailed deer (WTD) in the southeastern USA. Acute fatal HD is most often associated with northern latitudes, and results from experimental infection studies provide evidence for the role of innate host factors in HD resistance. In Georgia (GA), a consistent geographic pattern of mortality has prompted speculation regarding the potential role of spatial variation in host innate factors introduced to the state during extensive restocking efforts to reestablish the species following near extirpation in the early 20th century. This study estimated the population genetic structure of white-tailed deer in GA using mitochondrial and microsatellite markers, compared extant deer from GA and Wisconsin (WI) to identify residual genetics from source populations, and evaluated Georgia deer for disparities in allelic diversity at the major-histocompatability (MHC) class I heavy chain locus. Metapopulations across the state demonstrate varying degrees of genetic isolation, and two isolated populations (Blue Ridge Mountains, barrier islands; $p<0.05$ for mtDNA) are associated geographically with increased HD mortality reports. Of 16

mitochondrial D-loop control haplotypes identified in 21 Wisconsin WTD, 7 (43.8%) were identical to haplotypes found in GA WTD, and were associated spatially with counties where WI deer were introduced in the mid-1900s. No significant differences were found in allelic diversity at the MHC Class I heavy chain locus across sampled locations. In order to appreciate purportedly high level of spatial genetic heterogeneity in Georgia white-tailed deer attributed to extensive restocking, we compared genetic diversity and structure of white-tailed deer in two states. Mitochondrial sequences were compared between similarly sized areas of both Georgia (restocked) and Wisconsin (no restocking history). Although the number of haplotypes identified in each area were similar, they were significantly more variable in Georgia. Our findings suggest fine-scale genetic structure in GA WTD, influenced by introduction history, with isolated metapopulations corresponding geographically with areas of increased HD mortality. However, host-pathogen dynamics are complex, and host innate factors represent only a portion of potential variables influencing the geographic distribution of HD mortality in GA.

INDEX WORDS: White-tailed Deer, Population Genetics, Hemorrhagic Disease, Bluetongue, Georgia, Wisconsin, Mitochondria, Microsatellite, Major Histocompatibility Complex

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DEDICATION

For my parents. Mom, thanks for always believing I can do Anything, and Dad, for always thinking about The Logistics. This one's for you.

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CHAPTER I

INTRODUCTION

Hemorrhagic disease (HD) in white-tailed deer (WTD) is caused by infection with viruses of either of two closely related serogroups; bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHD) (Murphy, Howerth et al. 2005; Allison, Goekjian et al. 2010). While the etiology and pathogenesis have been studied extensively, population-level disease dynamics are not completely understood. Serologic surveillance and disease reports have consistently demonstrated a non-uniform geographical distribution of HD in WTD across North America. Generally, disease outbreaks in northern latitudes are infrequent and are characterized by severe clinical disease, while southeastern states report frequent, milder events (Davidson and Doster 1997; Gaydos, Crum et al. 2004). Clinical disease is nearly absent in the southwest (Stallknecht, Luttrell et al. 1996). In the state of Georgia, there is also a consistent geographical pattern of reported HD in WTD. Mortality has most often been reported in the Blue Ridge mountains and central piedmont, with sporadic events on barrier islands (Figure 3.1) (Nettles, Davidson et al. 1992).

Disease prevalence and severity in free ranging populations can be affected by a multitude of potential factors. These include, but are not limited to, population density, vector species composition and competence, viral strain and virulence, environmental factors affecting host and vector health, physiogeography, herd immunity, and innate host characteristics (Nettles and Stallknecht 1992). Previous studies have worked to elucidate the potential role of innate immunity in deer as a factor in HD resistance, and their results suggest an innate component to

HD clinical disease outcome (Gaydos, Davidson et al. 2002). The conclusion that host genetics represent a significant factor affecting the geographic pattern of HD in Georgia would require genetically distinct populations of WTD across the state. Due to the large size and mobility of this species, and population genetics studies in closely related species (*O. hemionus*), it is conceivable that an area the size of Georgia would not demonstrate the degree of genetic structure required to account for the distribution of HD mortality (Wayne, Pease et al. 2009). However, previous studies in WTD in the southeast have reported a high degree of population structure using both allozyme and microsatellite markers (Purdue, Smith et al. 2000; DeYoung, Demarais et al. 2002). These findings have been attributed to the extensive white-tailed deer restocking efforts during the early to mid-20th century, which introduced deer from northern, eastern, and southwestern states to mainland Georgia (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975).

The fate of these introduced animals and their potential influence on contemporary populations is under some debate. Some authors have suggested that deer introduced from northern latitudes, specifically Wisconsin, which was the source of hundreds of relocated deer in GA, did not survive in sufficient numbers to influence current herds due to HD mortality (DeYoung, Demarais et al. 2003). Although many reports suggest that the high spatial heterogeneity of southeastern WTD is attributable to 20th century restocking (Anderson, Honeycutt et al. 2002; DeYoung, Demarais et al. 2003), to our knowledge, no study has analyzed comparable populations from a restocked area and an area allowed to rebound without interference.

An additional genetic marker that has been suggested as a factor in disease resistance is the major histocompatibility complex (MHC). Loci in the MHC have been attributed to several

fitness characteristics in white-tailed deer and other species (Ditchkoff, Lochmiller et al. 2001; Eizaguirre, Yeates et al. 2009). An increase in allelic diversity at the MHC is purported to allow the individual to recognize a greater array of potential pathogens. MHC Class I has been analyzed in cattle (Birch, Murphy et al. 2006) and was chosen for this study due its localization in all cells, including endothelial cells, infection of which is central to clinical HD.

The overall aim of this study is to genetically characterize white-tailed deer populations of Georgia and estimate the potential role of host genetics in HD clinical disease at the population level. We will address 4 main questions: 1) What is the spatial extent of population structure in Georgia white-tailed deer, and does it correlate spatially with the prevalence of clinical hemorrhagic disease? 2) Where were deer introduced in Georgia, and is there evidence of shared genetics between current deer and their source population? Or did northern introduced deer succumb to HD or other factors before they could contribute genetically? 3) How diverse are Georgia white-tailed deer, and are their spatial differences in population heterogeneity that correlate with clinical HD prevalence? 4) Are there spatial differences in diversity at major histocompatibility complex loci, and do they correlate with HD prevalence? 5) How diverse are Georgia deer in comparison with a state that has not been restocked?

In this study we addressed these questions through analyses with the following objectives:

1. To estimate fine-scale population structure in Georgia white-tailed deer and identify correlations with spatial differences in HD mortality reporting.
2. To genetically characterize Georgia deer populations by calculating nucleotide diversity and heterozygosity indices and identify spatial correlations between diversity and HD mortality.

3. To determine whether current Wisconsin deer share mitochondrial haplotypes with deer in Georgia regions historically restocked with Wisconsin deer.
4. To identify spatial disparities in MHC Class I allelic diversity and compare them to the prevalence of HD mortality.
5. To confirm the effect of restocking efforts on genetic spatial heterogeneity in Georgia white-tailed deer through comparison of mitochondrial genetic structure in both Georgia and Wisconsin, where deer recovered from overhunting without interference.

Reports of mortality and morbidity attributed to hemorrhagic disease in Georgia deer was compiled from records at the Southeastern Cooperative Disease Study (SCWDS) and an index was created and mapped across the state to provide a means of comparison with spatial genetic data (objectives 1, 2, and 4). The genetic markers selected for use in estimating population structure (objective 1) included both cytosolic (mitochondrial D-loop control) and nuclear markers (microsatellites and MHC) as well as both presumably neutral loci (microsatellites and mitochondrial D-loop control) and loci under selection (MHC). By combining information from these three marker types, we provided a comprehensive picture of white-tailed deer phylogeography in the state. Diversity data from all three markers were calculated for population comparisons (objectives 2 and 4). Historical introduction data was screened for out-of-state sources, and was mapped across Georgia to identify areas potentially influenced by introduced deer (objective 3). We focused on Wisconsin reintroductions as this source population is sufficiently distant geographically to nearly eliminate the likelihood of shared mitochondrial haplotypes being due to shared ancestry independent of reintroductions. Mitochondrial sequence data was used to identify shared genetics between Georgia and Wisconsin to compared spatially to determine if shared haplotypes in Georgia occur in areas restocked with Wisconsin deer

(objective 3). The MHC Class I heavy chain locus was amplified and sequenced using 454 pyrosequencing to clarify all alleles present in each sampled population (objective 4). Mitochondrial sequence data was obtained from collaborators in Wisconsin to compare genetic heterogeneity and structure in a comparable area of each state to exemplify the differences between an anthropogenically restocked state and a state that recovered from severe population declines without human interference (objective 5).

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CHAPTER 2

LITERATURE REVIEW

HEMORRHAGIC DISEASE IN WHITE-TAILED DEER

Hemorrhagic disease (HD) is the most significant infectious disease of white-tailed deer (*Odocoileus* virginianus, WTD) in the southeastern United States (Nettles and Stallknecht 1992). Clinical HD is caused by viruses belonging to either of two closely related serogroups, epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) (Reoviridae: Orbivirus). Of the two serogroups, the EHD viruses are most often associated with disease in WTD (Nettles, Davidson et al. 1992), though clinical disease resulting from infection with either serogroup is indistinguishable without laboratory diagnostics (Nettles, Hylton et al. 1992; Murphy, Howerth et al. 2005).

History

Hemorrhagic disease has been recognized in white-tailed deer for over a century, though formal documentation and research has been limited to the last 40-50 years. In 1955, a mortality event involving over 230 confirmed deaths was reported in white-tailed deer in New Jersey. The total number of deaths was estimated to be 500-700 animals, constituting mortality rates exceeding 50% in some areas. At the time, biologists believed the event to be due to an emerging disease, and dubbed the associated syndrome "epizootic hemorrhagic disease (EHD)" (Shope, Macnamara et al. 1960). However, reports of similar events were found from as early as 1890, attributed to a wide range of ailments including blackleg, mycotic stomatitis, and hemorrhagic septicemia. In 1949-1954, deer mortality in the Southeast was attributed to a new deer pathogen,

dubbed “Killer X” (Nettles and Stallknecht 1992). These cases had numerous salient features in common, including consistent gross lesions, proximity of carcasses to water, and disappearance of the disease after the first frost (Nettles and Stallknecht 1992). Woodsmen had long since termed the condition in WTD “black-tongue”, presumably referring to the characteristic protruding darkened tongue of affected carcasses (Shope, Macnamara et al. 1960). Most early reports were from southern states, and subsequentlyreported in Washington State (1946, 1953), Missouri (1952-56), Michigan (1955), South Dakota (1956), and Alberta Canada (1962) (Shope, Macnamara et al. 1960; Fletch and Karstad 1971). By 1960, the etiologic agent had been identified as a filterable viral agent, and the first isolates were obtained from tissue of deer killed in epizootics in New Jersey (1955, EHDV-1), Alberta (1962, EHDV-2) and South Dakota (1956, although this isolate was lost) (Shope, Macnamara et al. 1960; Nettles, Hylton et al. 1992). By the early 1970s, a detailed disease pathogenesis had been proposed, ultrastructural examinations of the virus and its genome were published, and competent vectors (*Culicoides* spp.) had been experimentally identified (Tsai and Karstad 1973; Tsai and Karstad 1973; Fischer, Hansen et al. 1995).

Sporadic epizootics continued to be reported, including in West Virginia (1981, 1988, 1993), and Missouri (1988) (Fischer, Hansen et al. 1995; Gaydos, Crum et al. 2004). By 1990, the Southeastern Cooperative Wildlife Disease Study (SCWDS) had received 1608 reports of HD in 31 states, including 880 counties and parishes. Approximately one third of these reports (33.8%) were of deer mortality, while reports of chronic post-infection lesions comprised 55%. Overall, between 1955 and 1990, at least 45 mortality events were reported in wild ruminants that were confirmed as HD events by virus isolation (Nettles, Hylton et al. 1992).

Clinical disease

Clinical HD in deer may be peracute, acute, or chronic (Prestwood, Kistner et al. 1974; Nettles, Hylton et al. 1992). Clinical signs associated with acute cases of HD include erythema, facial edema, coronitis, lameness, dehydration, stomatitis, inappetence, lethargy, drooling, dyspnea, fever, mucosal congestion, recumbency, and terminal convulsions. A straw colored transudate is commonly found in thoracic, pericardial, and abdominal cavities. Peracute cases typically result in death 2-4 days following onset of clinical signs, and the primary gross finding in these animals is severe pulmonary edema. Animals with acute HD typically survive up to several days longer than peracute cases, and therefore may have gross lesions as described above as wells as erosion and ulceration of the dental pad, buccal papilla, and ruminal pillars (Shope, Macnamara et al. 1960; Karstad, Winter et al. 1961; Fletch and Karstad 1971; Quist, Howerth et al. 1997). If the animal survives infection, chronic lesions may include loss of rumen papillae, resulting in emaciation due to reduced rumen surface area and poor absorption (Couvillion, Nettles et al. 1981). The most commonly reported chronic lesion associated with HD is the disruption of the hoof wall, resulting in "sloughing hooves"(Nettles and Stallknecht 1992).

Host species

While white-tailed deer are the species primarily affected by HD, other species have been shown to be susceptible. Fatal infections of wildlife have been reported in mule deer (*Odocoileus hemionus*) and pronghorn antelope (*Antilocapra americana*), while bluetongue-like illness has been occasionally reported in elk (*Cervus elaphus*), bighorn sheep (*Ovis canadensis*), and bison (*Bison* bison) (Robinson, Hailey et al. 1967; Hoff, Richards et al. 1973; Dulac, Sterritt et al. 1992; Nettles, Hylton et al. 1992; Noon, Wesche et al. 2002). Of domestic species, sheep (*Ovis aries*) are most often associated with severe clinical disease, though typically due to infection

with BTV (Gibbs and Greiner 1994). Clinical disease in cattle (*Bos taurus*) and llamas is rare, but reported (MacLachlan, Barratt-Boyes et al. 1992; Yadin, Brenner et al. 2008; Meyer, Lacroux et al. 2009). Fatalities have been reported in dogs infected with BTV-contaminated vaccines (Akita, Ianconescu et al. 1994; Evermann, McKeiman et al. 1994).

Etiologic agents

Bluetongue virus is the prototype virus of the genus Orbivirus, and as a closely related virus, EHDV closely resembles its structure (Pierce, Balasuriya et al. 1998). Both EHDV and BTV are characterized by a segmented dsRNA genome, housed within a 3-layered icosahedral protein capsid. The genome is comprised of 10 linear segments, of which there is a single copy within each virion. The outer capsid is comprised of trimers of VP2 and Vp5. VP2 (encoded by genome segment 2) is the outermost protein, and the most variable in orbiviruses. This protein is most closely associated with viral entry into host cells, contains neutralizing epitopes, and controls serotype. VP5 (encoded by genome segment 6), also influences serotype, and has been associated with host membrane fusion (Ross-Smith, Darpel et al. 2009).

Worldwide, there are currently 10 EHDV serotypes recognized (serotypes 1-8, EHDV-318, and Ibaraki virus (IBAV), although it has been proposed that these be condensed further to 7 serotypes (including EHDV-3 into the EHDV-1 serotype, EHDV-318 into EHDV-6, and IBAV into EHDV-2) (Anthony, Maan et al. 2009; Allison, Goekjian et al. 2010). Currently 3 EHDV serotypes (EHDV-1,2 and 6) and 6 BTV serotypes (BTV-1,2,10,11,13, and 17) are endemic to North America (Stallknecht, Nettles et al. 1995; Johnson, Ostlund et al. 2006; Allison, Goekjian et al. 2010).

Vectors

Transmission of EHDV and BTV is primarily by *Culicoides* spp. (Diptera: Ceratopogonidae). The species identified as the primary vector in the US is *C. variipennis (Smith, Stallknecht et al. 1996). Culicoides variipennis* has been reported in mainland Georgia as well as most of the southeastern US (Stallknecht, Kellogg et al. 1991; Smith, Stallknecht et al. 1996). *Culicoides insignis* has also been confirmed as a competent vector for HD viruses in domestic ruminants in Florida, and may transmit EHDV or BTV to free-ranging ruminants elsewhere (Smith, Stallknecht et al. 1996).

Ossabaw Island, which experiences sporadic HD epizootics is also home to *Culicoides*, though only *C. furens* had been identified by the late 1980s (Stallknecht, Kellogg et al. 1991). However, surveys of midges in coastal Georgia including Chatham County (which includes Ossabaw Island offshore), have identified *C. variipennis* (Stallknecht, Kellogg et al. 1991).

GEOGRAPHIC PATTERN OF HD IN THE US

The distribution of HD in the US is geographically non-uniform. Generally, clinical disease occurs most often in the southeast, Midwest, and Pacific Northwest, with few reports of clinical HD in the southwest and northeast (Nettles, Davidson et al. 1992; Davidson and Doster 1997). Serologic surveys have demonstrated seroprevalence of antibodies to HD viruses nearing 100% in the southwestern states, including Texas. These areas may represent areas of enzootic stability, where EHDV and BTV exist in a near perfect host-virus relationship (Stallknecht, Luttrell et al. 1996; Gaydos, Davidson et al. 2002).

The first reported large scale outbreak of HD in the southeastern US was during the fall of 1971, involving deer in North Carolina, Florida, Georgia, Tennessee, Kentucky and Virginia.

Although one penned deer herd in Mammoth Cave National Park, Kentucky, experienced approximately 60% mortality, elsewhere mortality rates were variable, and correlated with deer density (Prestwood, Kistner et al. 1974). Clinical disease consistent with HD was reported annually in the southeastern US from 1971 through 1980, when another large outbreak was reported, involving 156 counties across Alabama, Florida, Georgia, Mississippi, Missouri, North Carolina, South Carolina, and Virginia (Couvillion, Nettles et al. 1981). Overall, historical data from the southeastern US support the hypothesis that epizootics in the Appalachians tend to be isolated, sporadic, and severe, while disease occurrence in coastal areas tends to be frequent and mild (Gaydos, Crum et al. 2004). Areas in between these extremes vary in disease prevalence.

A means of estimating viral prevalence and host exposure is serologic surveillance of healthy animals. Researchers and biologists at SCWDS and elsewhere have reported significant variation in neutralizing antibodies to EHDV and BTV serotypes that vary by latitude, physiogeography, and year (Stallknecht, Blue et al. 1991). In years following an epizootic, serum neutralizing antibodies tend to wane in the population, being restricted to older age classes (Stallknecht, Kellogg et al. 1991). In the years 1981-1989, an extensive serologic study of Georgia found a precipitous drop in seroprevalence of antibodies to HD viruses in barrier island populations, which were 68% in the fall of 1981 and 3% in 1989 (Stallknecht, Blue et al. 1991). In general, the overarching pattern of EHD seroprevelance demonstrates low circulating antibodies in mountain and barrier island populations, and variable (approximately 5-65%) levels in the piedmont and coastal plain (Stallknecht, Blue et al. 1991).

The consistent geographic patterns of HD activity in the US may be due to a number of contributing factors, including characteristics intrinsic to the vector and environment. Differences in the seasonality, abundance, and vector competence of resident *Culicoides* may

result in differences in viral exposure between geographically disparate WTD populations. For example, though clinical HD is rare in the Caribbean, Central America, and Florida, surveillance for bluetongue virus has shown the agents to be commonplace, and surveys for the vector have found the presence of *Culicoides* year-round. In this scenario, it is likely that fawns are exposed to virus while still protected by maternal antibodies, and infection results in a "vaccinating" dose (Nettles, Davidson et al. 1992).

Geographic variation in vectors, virus strain and virulence, and herd immunity have all been considered to influence the spatial variation in HD activity in the US. It has been hypothesized that these factors may have influenced genetic selection in white-tailed deer, resulting in genetic factors affecting disease susceptibility between populations. A 2002 study compared the outcomes of experimental infection between animals collected at sites both within and outside of known endemic areas. Fawns were collected from Pennsylvania (*O. v. borealis*) and Texas (*O. v. texanus*), representing historically epizootic and endemic areas, respectively. Once cleared of maternal antibodies, the deer were infected with EHDV-1 and EHDV-2 and monitored. Although both groups developed viremia, the PA fawns developed higher viral titers more quickly than TX fawns, and PA fawns had greater lymphopenia and more severe clinical disease. All of the PA fawns (n=5) infected with EHDV-1 died and 20% (n=5) of the PA fawns infected with EHDV-2 died. None of the TX fawns succumbed to the illness (Gaydos, Davidson et al. 2002). The results of this study suggest that a component of immunity to HD viruses in southern deer (*O. v. texanus*) may be innate, independent of circulating antibodies. These experiments compared deer from geographically distant areas, which would be assumed to be genetically distinct. In order for differences in host genetics to play a significant role in the

spatial pattern of HD in Georgia, fine-scale genetic structuring would be required to result in genetically distinct populations.

POPULATION GENETICS IN WHITE-TAILED DEER

Geographic population structure is dependent upon restriction of gene flow, and is influenced by species dispersal, mating strategies, migration, and other factors (Freeland 2005). While migration in white-tailed deer (WTD) is typically restricted to winter foraging, and is therefore of no consequence in the south, WTD are large and highly vagile, and are present in abundance across a continuous range through both mainland Georgia and its associated barrier islands. These characteristics are not conducive to the restrictions in gene flow that delineate populations and metapopulations in a continuous species range. Here we will review other species characteristics that affect gene flow and the formation of potential metapopulations within this continuous range.

White-tailed deer populations are most successful in areas containing at least patchy woodlands and early successional habitat. However, the species is a broad generalist and exists exceedingly well in peridomestic environments, providing adequate fawning cover and reliable food sources (Demarais, Miller et al. 2000; Miller, Muller et al. 2003). In the non-breeding seasons, the home range of an adult female is about 50% that of an adult male in the same area. A study of WTD home-ranges in Georgia reported an average of 140.9ha in summer and 167.4ha in winter for females, and 235.7ha for summer and 206.1ha for winter in adult males (Rogers 1996). During the breeding season, males may range widely in search of estrous does, and females, too, may make brief "breeding excursions" outside of their normal range if no available males are present (Miller, Muller et al. 2003). Once they reach sexual maturity, yearling bucks

typically leave their dams and form bachelor groups. This may be initiated by aggression from both the dam and her close relatives. Yearling buck dispersal usually ranges from 3-10km, though distances of ≥150km have been reported. Female dispersal also occurs, though it is more dependent on competition for food sources and suitable fawning habitat. In areas with patchy forest habitat and plentiful nutrition, female dispersal rates may be less than 5%, whereas in intensely farmed areas, female dispersal may approach 50% (Miller, Muller et al. 2003).

White-tailed deer utilize a tending-bond mating system, where a buck will court an individual doe for up to several days to breed. Adult males actively pursue and fight for access to estrous females, and although this system is polygamous, it is unlikely that males in free-ranging populations are able to effectively defend more than one female at a time. This limits the likelihood that a single male will dominate genetic contributions to the next generation. Evidence of multiple paternity in litters also signifies the influence of alternative breeding strategies in WTD (DeYoung, Demarais et al. 2002; DeYoung, Demarais et al. 2009). Female WTD produce an average of two fawns each spring, which will generally remain with their dams until their second fall. In areas where fawns have access to excellent nutrition, individuals of either gender may reach sexual maturity in their first year, though more commonly they breed first as yearlings (Miller, Muller et al. 2003).

Dispersal of white-tailed deer in Georgia is unlikely to be completely precluded by barriers, natural or anthropomorphic. Although mountain ranges and wide water bodies may reduce deer movements, there are few obstacles that deer will not readily cross, especially during the breeding season. Human barriers are rarely a complete deterrent; from a standing position, an adult white-tailed deer can leap a 7ft fence, and with a running start, may clear over 8ft. White-tailed deer are also adept swimmers and will ford rivers or cross inter-coastal waterways to

populate barrier islands (Baker 1984; Miller, Muller et al. 2003). Despite the apparent vagility of deer, studies in similar species have shown that landscape features do restrict gene flow. A study in roe deer found that while a single barrier (canal, highway, etc.) was not sufficient to result in genetic differences between deer, compounded barriers (ex. a canal, highway, and housing development together) resulted in detectable genetic differences in deer on opposing sides (Coulon, Guillot et al. 2006).

In this study, we will be analyzing mitochondrial DNA, which will be discussed in later sections of this review. One of the assumptions of analysis of this marker is that there is no heteroplasmy, meaning that the individuals studied harbor only 1 mitochondrial genome. While heteroplasmy is rare, it has been reported in mice and humans, and has increased occurrence in hybrids (Ballard and Whitlock 2004). Hybridization between mule deer and white-tailed deer is rare in free-ranging populations, though captive animals have produced viable hybrids (Cronin, Vyse et al. 1988). However, mule deer are not present in the two states studied in this project, and therefore, we continue to assume zero to insignificant heteroplasmy in our samples.

Comparisons between deer in Georgia and Wisconsin require comparison between two described subspecies. However, subspecies delineations are based on phenotypic variation, which is notably disconnected from genotypic variation (Humphries and Winker 2011). Taxonomists have described 17 subspecies of WTD in North America north of Mexico, with a further 13 subspecies in Mexico and Central America, and 8 in South America (Baker 1984; Geist, O'Gara et al. 2000; Miller, Muller et al. 2003). While these subspecies have not been critically examined for genetic differentiation, two subspecies in the US have been officially listed as endangered. One of these, the Columbian white-tailed deer (*O. v. leucurus*), is isolated in the river basins of Oregon in the western US. In a study of allozyme allelic frequencies, it was

suggested that the Columbian white-tailed deer sampled may not have been unique enough to warrant subspecies status (Gavin and May 1988), though little work has been published demonstrating genetic parameters for subspecies delineations. White-tailed deer subspecies have been taxonomically differentiated based on geographic location and morphological characteristics including body mass, pelage color, cranial dimensions and antler size and shape (Miller, Muller et al. 2003; Seabury, Bhattarai et al. 2011). However, these attributes may be significantly affected by habitat features such as soil region and nutrition quality, and therefore may not reflect genetic differentiation (Strickland and Demarais 2000). The subspecies confusion in whitetails is further compounded by the extensive restocking efforts implemented during the early to mid-1900s following the near extirpation of white-tailed deer throughout much of North America. The remixing of animals from geographically distant areas may have resulted in loss of readily detected regional differentiation required for subspecies definitions (Geist, O'Gara et al. 2000). Details of the restocking effort and its effects on Georgia WTD populations will be discussed further in later sections. According to published documentations, the subspecies present throughout mainland Georgia (and most barrier islands, including Ossabaw Island) and most of the southeastern US is *O. v. macrourus*, with an additional subspecies, *O. v. nigribarbis* reported on Blackbeard Island, off the Georgia coastline. The subspecies inhabiting Wisconsin is *O. borealis* (Baker 1984; Demarais, Miller et al. 2000; Rue 2004).

RESTOCKING OF WHITE-TAILED DEER IN GEORGIA (1928-1979)

By the early 20th century, WTD were nearly extirpated from the southeastern US, following decades of unregulated hunting and, in heavily farmed areas, indiscriminate killing to prevent crop consumption. The actions implemented to protect and augment failing WTD populations

may represent the largest and most successfully executed conservation effort ever attempted in a large mammal, and its effects are still apparent today (DeYoung, Demarais et al. 2003).

Commercial hunting and the unregulated harvest of WTD for meat and other products was not effectively stopped until after 1900, with the signing of the Lacey Act (Musgrave 1998; Demarais, Miller et al. 2000). However, while small restocking efforts in some areas began in the late 1800s, large scale reintroduction efforts did not begin until Pittman-Robertson funds, from the Federal Aid in Wildlife Restoration Act signed in 1937, made such efforts fiscally feasible (Demarais, Miller et al. 2000; Miller, Muller et al. 2003). In 1938, the Georgia Game and Fish Commission began establishing wildlife management areas to protect deer, and soon after, large scale restocking efforts were initiated. With federal and state regulations protecting deer from harvest, and the local extinction of natural predators (wolves and mountain lions), white-tailed deer flourished, and the first regulated hunting season was held a mere 12 years after the programs were implemented (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975). Although white-tailed deer in Georgia neared extinction by the end of the 19th century, by 1975 the estimated population in Georgia was estimated to be 200,000-250,000 animals (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975), and numbers today are thought to exceed those present in pre-colonial times (Demarais, Miller et al. 2000). Contemporary wildlife managers now face the management of overabundant deer, which often represents their greatest challenge (Davidson and Doster 1997).

The end result of deer restocking efforts in Georgia was a rapid expansion of both severely reduced and isolated native populations along with the addition of introduced deer from such distant areas as Wisconsin, Texas, Maryland, Virginia, North Carolina, and Kentucky, with additional relocations between areas of the state including from the barrier islands, where native

deer populations remained protected due to their relative inaccessibility. The total recorded number of white-tailed deer restocked in Georgia was 3741 animals, 1892 of which came from outside the state. Tables 2.1 and 2.2 include a summary of the data reported by the Federal Aid in Wildlife Restoration State Game and Fish Commission of Georgia compiled in 1975, including the sources of restocked deer, and the site and date of their release (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975).

POPULATION GENETIC ANALYSES

In order to characterize Georgia white-tailed deer populations, we will analyze three marker types: mitochondrial DNA (mtDNA), microsatellite markers, and the MHC Class I heavy chain locus. The MHC will be discussed further in the next section.

Mitochondrial DNA

One of the most common means of studying wildlife genetics today is through sequencing of mitochondrial gene markers including portions of the cytochrome b (*cyt b*), cytochrome c oxidase 1 (COI), and the mitochondrial D-loop control region (D-loop) (Dawnay, Ogden et al. 2007; Dalton and Kotze 2011; Ogden 2011). Additional mitochondrial markers, including rRNA subunits, have also been extensively used (Bellis, Ashton et al. 2003; Sahajpal and Goyal 2010; Rojas, Gonzalez et al. 2011). The circular, double stranded mitochondrial genome is miniscule, consisting of only 15,000-17,000bp (16,569bp in humans), roughly 1/10,000 the size of the smallest animal nuclear genome (Ballard and Whitlock 2004; Karlsson and Holmlund 2007). This cytoplasmic genome encodes 37 genes: 24 genes encoding translation machinery (22 tRNAs and 2 rRNAs), and 13 genes encoding subunits of the electron transport chain. Early RFLP-based research analyzing mtDNA reported disproportionately high mutation

rates, stimulating further development of mtDNA as a molecular tool. In 1989, the publication of highly conserved mtDNA primers simplified the direct sequencing of mitochondrial genes across numerous species. Due to its lack of recombination, maternal mode of inheritance, and relative ease of amplification, mtDNA is now targeted in studies aiming to answer a wide variety of ecological, phylogenetic, taxonomic, and phylogeographic questions (Ballard and Whitlock 2004). In this study, we will analyze sequences derived from the mitochondrial D-loop control region (mtDNA CR). The mtDNA CR is hyper-variable, mutating up to 5 times faster than the remaining mitochondrial genome (Wu, Wan et al. 2005). For this reason, this region is often used to delineate between populations that are have recently separated (Ballard and Whitlock 2004).

Microsatellites

Microsatellite markers (aka short tandem repeats, STRs) consist of numerous tandem 2-6bp repeats surrounded by conserved flanking regions. These repeats are found throughout the nuclear genome, and are often conserved between closely related species. They are so called 'micro' following the previous discovery of minisatellites, which are much longer (repeats of 33bp segments or longer) (Jeffreys, Wilson et al. 1985). Microsatellites vary in length due to differences in repeat numbers, which are hypervariable due to the ease of stutter error in replication machinery. Differences in size make alleles easily differentiated without the need for direct sequencing. Unlike mtDNA markers, which rely on phylogenetic comparisons of haplotype sequence and frequency at a single locus, microsatellite data is typically compiled across several analyzed markers, resulting in a multilocus genotype for each individual.

Marker panels are published for numerous species, but in uncommon and non-model species, it is often necessary to identify markers *de novo*. The development of microsatellite markers

involves the identification of repeat motifs using bead capture technology (Jones, Levine et al. 2002). Once microsatellites are identified, they are amplified using primers designed to be complementary to their conserved flanking sequences (Poetsch, Seefeldt et al. 2001). Microsatellites are selected based on ease of amplification, polymorphism, and simplicity of allele determination. Allele identification can be somewhat subjective, and therefore it is important to work with markers that provide consistent genotyping data, lacking complicating characteristics (ex. stutter peaks). Typically, tetranucleotide repeats are simpler to genotype than dinucleotide repeats (Jones, Levine et al. 2002; Dawnay, Ogden et al. 2008).

While it may be best to identify species-specific microsatellite markers, development methods can be time consuming and cost-prohibitive for laboratories with limited facilities. Alternatively, amplification of DNA using microsatellite loci designed for closely related species frequently results in viable allele data, though errors may result from using non-specific primers (Hoff-Olsen, Jacobsen et al. 2001; Poetsch, Seefeldt et al. 2001).

Nuclear vs. cytoplasmic markers

Nuclear markers (ex. microsatellites, minisatellites, MHC, SRY, etc; nDNA) and cytoplasmic markers (ex. mitochondria (mtDNA), chloroplasts) have both been used extensively in phylogenetic studies. Opinions differ widely as to which type is ideal for population analyses, and thus most agree that both types should be analyzed before conclusions may be made regarding population structure. In order to understand the differences in value of these markers, it is important to understand some basic principles regarding their inheritance and how they come to represent populations. These principles include the concepts of genetic drift, and effective population size (Freeland 2005).

The two primary differences between nuclear and mitochondrial DNA are that the latter is both uniparentally inherited, and haplotypic (Ballard and Whitlock 2004). Both of these features significantly alter the dynamics of gene flow and allele frequency changes across a population. Most phylogenetic analyses are based at least in part on allele or haplotype frequencies within and among populations. Significant differences in allelic frequencies (as determined by Wright's F_{ST} and similar statistics) is indicative of reduced or absent gene flow, and suggests sampling from distinct populations. Allele frequency of neutral markers (ex. microsatellites and mitochondrial D-loop control region) changes over time between genetically isolated populations through genetic drift. Genetic drift is the process by which the allelic (or haplotypic) frequencies in a population change over time due to random sampling between generations. These changes occur because the alleles passed in a population from generation to generation are not constant, due to reassortment in meiosis as well as variable reproductive success between individuals (Freeland 2005).

The effects of genetic drift are more rapidly apparent in small populations with limited gene flow, though the effects will eventually be significant in large populations as well. As such, this process is closely linked to the determination of effective population size (N_e), which is one of the cornerstone theoretical measures of population structure (Freeland 2005). Mitochondrial DNA, because of its uniparental mode of inheritance and haplotypic qualities, has an effective population size approximately ¼ that of nuclear DNA. This disparity results in more rapid fixation of alleles in mtDNA due to genetic drift, and therefore more rapid differentiation between mtDNA haplotype frequencies in recently isolated populations than would theoretically be observed in neutral nDNA markers.

These generalizations assume that approximately half of the breeding populations are female, which may not be the case, especially in polygamous species. In these species, where dominant males may have significantly greater reproductive success than others, effective population size (attributed to nDNA, $N_{e,nuc}$) will be decreased, because a reduced number of males are contributing to the effective gene pool. In these situations, the mitochondrial N_e is not affected, since males do not contribute to mtDNA diversity. Although in general the drift in mitochondrial genes is more rapid than in nuclear genes, if the effective number of males in a population is less than $1/7^{th}$ of the number of effective females in that population, then the genetic drift in nuclear genes would be greater than the drift in mitochondrial genes, and may therefore be of equal or greater value in elucidation of recent genetic structure (Ballard and Whitlock 2004; Freeland 2005).

In general terms, genetic diversity is achieved by sexual reproduction, chromosomal recombination, independent assortment during meiosis, and random mutation. While all of these influence nuclear markers (although recombination of short tandem repeats (microsatellites) is assumed to be negligible), only random mutation affects mtDNA (in the absence of heteroplasmy). However, the mutation rates of mitochondrial genomes are higher than those of nDNA, with reports as high as 2% per million years. This is approximately 10 times faster than rates reported for nDNA, though this rate will differ in areas of the genome under even the slightest amount of selection pressure (Ballard and Whitlock 2004).

Another benefit of mtDNA markers is their consistent and robust usability, especially in wildlife research. Unlike nuclear DNA, which exists as only one set of diploid copies present per cell, mitochondrial DNA is present in each of potentially thousands of cytoplasmic mitochondria per cell (the actual number varies with cell type). This abundance makes successful DNA

extraction possible in the highly degraded samples common in wildlife research, which often utilizes samples like scat, shed hair, or carcasses as sources of DNA (Amorim 2010).

Limitations of mtDNA

Mitochondrial DNA has several potential limitations as a molecular tool. For one, although it is assumed that there is no recombination in mitochondrial genomes, there have been reports in the literature which suggest that such events are possible in some species, including *Drosophila* and humans (Ballard and Whitlock 2004). For mitochondrial recombination to occur there must be a proportion of the population that are heteroplasmic, meaning they contain more than one mitochondrial haplotype. This is typically due to paternal leakage, wherein mitochondria from the fertilizing sperm survived in the ovum, resulting in biparental origin of the fetus' mitochondria and their genomes (Ballard and Whitlock 2004). Other potential sources of error include somatic mutations and pseudogenes. Somatic mutations are changes in mtDNA haplotype that occur during an individual's lifetime and are typically tissue specific. These are difficult to differentiate from germline mtDNA haplotypes if they are present in the majority of cells sequenced for a DNA sample. Conversely, nuclear markers, like microsatellites, have been shown to be generally stable across tissues, demonstrating limited somatic mutation (Hoff-Olsen, Jacobsen et al. 2001). The remaining sources of potential error, nuclear pseudogenes of mtDNA origin (NUMTS), are non-encoding regions which may amplify along with mtDNA (Ballard and Whitlock 2004). In these instances, as with heteroplasmy, multiple haplotypes may be present in a single individual. This may result in inexplicable failure of sequencing due to the mixture of submitted alleles, or bases identified equally as two different nucleotides. For this study, all ambiguous base calls were identified with degenerate nucleotides designations (N) to diminish the effects of multiple potential alleles. Sequences with ambiguous nucleotides at informative

sites were removed from analysis, as they cannot be definitively assigned a haplotype. This protocol should limit error due to potential heteroplasmy, NUMTS, and somatic mutation (unless the somatic mutation is present across all cells sequenced).

A REVIEW OF PHYLOGENETIC STUDIES IN DEER

Although white-tailed deer are one of the most widely distributed large mammals in North America, little sequence data has previously been available for this species. The complete WTD mitochondrial genome has recently been sequenced, in addition to over 10,000 single-nucleotide repeat (SNP) loci, which significantly expands the molecular resources available for deer genetic research (Seabury, Bhattarai et al. 2011). However, despite the obvious limitations, there have been numerous studies attempting to elucidate genetics in WTD and a myriad of other related species.

In the 1980s, researchers at the Savannah River Ecology Laboratory in South Carolina published several works involving genetic studies in WTD. These examined over 36 allozymes using starch gel electrophoresis. Their studies found disproportionately high levels of heterozygosity and overall genetic diversity in WTD as compared to other large mammals (Smith, Baccus et al. 1984; Breshears, Smith et al. 1988). A later study published data from 6 populations from coastal GA and South Carolina (SC), analyzing both an allozyme panel and the mtDNA CR. Mitochondrial data was derived using PCR-RFLP techniques instead of direct sequencing. Their findings suggest high genetic variability as well as a surprising degree of spatial heterogeneity, which was unexpected due to the highly mobile nature of this species. These authors attributed these findings to philopatry in female WTD, with minimum doe

dispersal (Purdue, Smith et al. 2000). Similar results were obtained from analysis of allozymes in New York deer, also attributed to female philopatry. These authors also report a deviation from Hardy-Weinburg equilibrium (HWE) resulting in heterozygote excess, which they attributed to turn-over in dominant males between years (Mathews and Porter 1993).

A large scale analysis of the phylogeography of mule deer in the western US analyzed a panel of 18 tetranucleotide repeat microsatellite markers and the mtDNA CR, identifying 5 genetic clusters across California (Pease, Freedman et al. 2009). Another study examined mule deer samples from across the species' range, analyzing both the *cyt b* and CR regions of mtDNA. This identified glacial refugia likely utilized by the species during the last glacial maximum (LGM). Twelve haplogroups were identified through network analysis, though estimation of fine-scale phylogeography was not possible (Latch, Heffelfinger et al. 2009). Mitochondrial D-loop control region sequence has also been used to differentiate subspecies of Chinese sika deer, which exist in geographically isolated areas (Wu, Wan et al. 2005).

The European roe deer, like the WTD, has undergone a great deal of anthropogenic dispersal during extensive restocking and reintroduction efforts. A phylogenetic analysis of this species examined the mtDNA CR (704bp) as well as 11 microsatellite markers. Three haplogroups (161 total haplotypes) were identified across southern Europe (Randi, Alves et al. 2004).

Previous studies comparing current genetic parameters and structure in introduced WTD have utilized several methods. An allozyme study evaluating deer across the southeastern US found associations between populations restocked from the same source populations, and no correlation between geographic and genetic distance in reintroduced populations, suggesting residual effects of anthropogenic migrations overriding current gene flow (Leberg, Stangel et al.

1994). Reintroduced white-tailed deer populations in Mississippi (MS) were evaluated using a panel of 17 microsatellites developed for white-tailed deer, a subset of a published set of 21 (Anderson, Honeycutt et al. 2002; DeYoung, Demarais et al. 2003). All markers were found to be polymorphic, with 240 alleles identified across all 17 markers, in deer from 16 populations across the state and three outside populations. Two loci-population combinations were monomorphic, and tests for HWE found deviations in 13 of 19 total populations, each having 1-3 loci not in equilibrium. The results of this study suggest population bottlenecks consistent with their demographic history, though this was not consistent in all populations. Overall, MS WTD populations have high genetic diversity, higher than levels found in North American elk, which have similar histories of exploitation, reintroduction, and population expansion. Current sub-populations in MS share similar genetics with founder populations, demonstrating lasting effects from reintroductions in the early 1900s (DeYoung, Demarais et al. 2003). These authors found no link between populations heavily restocked from Wisconsin sources, and concluded that deer from that state had little influence on current genetics. They attributed this to the potential effects of climate and pathogens (ex. HD) on introduced deer (DeYoung, Demarais et al. 2003). However, they did not sample WI deer during their study to allow for more robust conclusions regarding foundation stock.

To our knowledge, this study is the first to analyze WTD population structure across the state of Georgia, as well as the first to examine both mtDNA and microsatellites in Georgia deer. Previous studies examining current WTD populations following the reintroduction of WTD in other areas of the southeast examined only microsatellites, and did not compare data from WI source populations to determine residual genetics. This is also the first study, to our knowledge, to analyze MHC alleles in GA WTD, especially in comparison with HD susceptibility.

THE MAJOR HISTOCOMPATABILITY COMPLEX

The major histocompatibility complex (MHC) is composed of a large collection of genes comprised of two distinct but homologous groups, MHC Class I and II (MHC I and MHC II). The MHC Class I heavy chain locus of white-tailed deer will be analyzed in this study to determine if there are differences in levels of MHC diversity between populations in Georgia, and if a reduction in diversity is associated with regions historically susceptible to HD. Analysis of the sequence of MHC alleles in these populations will provide further information regarding population subdivisions, and could potentially identify alleles associated with resistant populations.

MHC function

The MHC was given its name during its discovery as a key determinant of tissue graft acceptance in human patients, and is an important component of the innate immune system. The function of the MHC is to bind short polypeptide sequences found in the cell and display them on the cell surface. These protein fragments may be derived from the cell itself (which may be altered in neoplastic cells and thus no longer 'self') or from proteins associated with intracellular or phagocytosed pathogens and parasites. Cells of the immune system, which differ depending upon the class of MHC involved, assess the displayed protein fragments. If these are determined to be non-self, actions are taken to limit spread of the pathogen or cancerous cells identified. The specifics of this general process differ between MHC I and II (Cresswell 1994; Abbas and Lichtman 2003).

MHC I

MHC I molecules are present in all somatic cells, and their primary function is to alert the immune system to the presence of infected or neoplastic cells. In practical terms, this allows all

somatic cells to become antigen presenting cells (APCs). The loading of peptides in the MHC I pathway occurs on the inner surface of the endoplasmic reticulum (ER), and amino acid segments are usually derived from products produced by cellular machinery. As proteins undergo routine degradation by the cell's proteasome, fragments are sequestered into the ER through a membrane bound transporter molecule (TAP). Peptides are recognized by the broad specificity of the variety of expressed MHC molecules, and are bound. These complexes make their way to the cell surface through secretory molecules packaged in the Golgi apparatus. Once on the surface, the MHC and bound peptide are each recognized by receptors on cytotoxic T lymphocytes (CD8+) (CTL). The T-cell receptor (TCR) has components that recognize both the self MHC and the bound peptide. If the CTL recognizes the peptide as non-self, it will initiate the cytolytic properties of these cells. Here, the CTL also has the opportunity to recognize non-self MHC, as would be the case in a tissue graft. As such, the foreign MHC becomes an antigen itself, and initiates cytolytic pathways (Abbas and Lichtman 2003).

MHC II

MHC II molecules are expressed in phagocytic cells, including macrophages, dendritic cells, and B-cells. In this pathway, the proteins that are degraded to provide segments for presentation are present within the phagosome of the cell. Therefore, as opposed to the cytosolic localization of proteins in MHC I presentation, these proteins were phagocytized by the cell, and likely originated from whole phagocytosed virus, bacteria, or other parasites circulating in blood or tissues. The MHC II molecule is present in the membrane of the phagosome, and proteins are degraded by proteases therein. Once bound to protein, the MHC II molecules are presented on the cell surface. Here they are recognized by T-helper (CD4+) cells, and if the bound peptide is non-self, the phagocyte is activated and its contents destroyed (Abbas and Lichtman 2003).

MHC gene expression

The power of the MHC lies in its ability to recognize a wide array of amino acid sequences to be presented to the immune system. However, unlike antibody or TCR diversity, MHC diversity is hardwired and is therefore finite for a given individual (Oppelt, Wutzler et al. 2010). A means by which the genetic information of an individual is maximized is through the multivariate structure of the MHC molecules.

The MHC encodes over 100 genes expressing predominantly immunological molecules (Oppelt, Wutzler et al. 2010). In humans, this locus comprises a large segment (about 3500kb) on the short arm of chromosome 6. Crossovers within this segment between homologous chromatids occur during approximately 4% of meiotic events (ie, the human MHC locus extends over 4 centimorgans). The MHC class I genes are located at the most telomeric of these loci, while the MHC class II genes are the most centromeric (Abbas and Lichtman 2003).

MHC nomenclature

The accepted nomenclature of MHC genes and molecules is complex, due to a long history of study in both human and mouse models. For the most part, human MHC molecules are called 'human leukocyte antigens (HLA)'. A similar scheme is used to name MHC genes in model animal species, for example bovine leukocyte antigens (BoLA) in cattle (Miyasaka, Takeshima et al. 2011), feline leukocyte antigens (FLA) in cats (Kennedy, Ryvar et al. 2002), and dog leukocyte antigens (DLA) in domestic canines (Debenham, Hart et al. 2005). In other species, the nomenclature is derived from the binomial species name, such that the DRB1 gene in domestic sheep (*Ovis aries*) is termed *Ovar-DRB1* (Ballingall and Tassi 2010). Likewise, the MHC DRB gene in white-tailed deer (*Odocoileus virginianus*) is termed *Odvi-DRB* (Ditchkoff, Lochmiller et al. 2001).

The MHC I molecules are encoded by the human *HLA-A, HLA-B, and HLA-C* genes, and are homologous to the *H-2K, H-2D* and *H-2L* genes in mice (Abbas and Lichtman 2003). MHC II genes are broadly categorized as *HLA-DR, HLA-DQ*, and *HLA-DP*, though each of these is further divided into individual loci, A and B, which are again subdivided numerically. For instance, in humans there are nine *HLA-DRB* loci, *HLA-DRB1-9*. Of these, *HLA-DRB2, 6, 7, 8* and *9* are pseudogenes, while the remaining 4 loci are functional. The *HLA-DRB1* locus, the most centromeric of these loci, is present in all human haplotypes and is the most polymorphic *DRB* locus (Kenter, Otting et al. 1992).

The set of MHC alleles encoded on a single chromosome is called an 'MHC haplotype', while the complete stock of MHC alleles present in an individual may be called their 'MHC genotype'. In genetics, the term 'haplotype' is most often associated with haplotypic alleles, for example mtDNA and X-chromosome loci in males. However, the term refers to the sequence identity of any section of directly linked DNA, as is the case with the long MHC locus (Abbas and Lichtman 2003).

Studies in MHC genetics

In recent years, there has been much discussion over the diversity of MHC genes and the influence this may have on species survival (Eizaguirre, Yeates et al. 2009; Miyasaka, Takeshima et al. 2011). MHC diversity has been studied in many vertebrate species in association with disease outcome and host-parasite interactions, with the hypothesis that low MHC polymorphism may make a population particularly vulnerable to infection. Indeed, the influence of MHC genotype on susceptibility to infection has been demonstrated in several vertebrate species (Wegner, Kalbe et al. 2006). However, empirical data associating overall

species viability and reduced MHC diversity have thus far proved equivocal (Babik, Pabijan et al. 2009).

Pathogen-mediated selection for MHC diversity is likely through heterozygote advantage (overdominance) and advantage to carriers of rare alleles. Both instances provide the widest possible repertoire for an individual, allowing the recognition of the greatest variety of antigens (Ekblom, SÆTher et al. 2007; Mona, Crestanello et al. 2008). At the population level, maintenance of both high diversity and retaining rare alleles provides increased survivability for a group to survive an introduced pathogen or parasite (Babik, Pabijan et al. 2009; Miyasaka, Takeshima et al. 2011). This balancing selection would theoretically result in selection for within-population MHC diversity greater than the diversity apparent in the rest of the genome (Bryja, Charbonnel et al. 2007), which has been demonstrated in numerous species, even those that have experienced recent bottlenecks or overall population declines (Aguilar and Garza 2006).

It is generally assumed that the selection pressure driving MHC diversity is pathogen-mediated (Ekblom, SÆTher et al. 2007; Mona, Crestanello et al. 2008). However, it has also been suggested that MHC loci are under direct sexual selection, based on theories of "good genes advertising". Studies of mate choice in a number of species have been linked to MHC genes. For example, olfactory mate choice in humans, mice, and stickleback fish has been shown to be MHC-linked. Secondary sexual traits that influence mate selection have been correlated with MHC genes, including the snood in turkeys, throat color in sticklebacks, and antlers in deer (Ditchkoff, Lochmiller et al. 2001; Eizaguirre, Yeates et al. 2009).

Analyzing MHC alleles is not as straight forward as in microsatellites, where allele sequence is assumed to be linked to its length in base pairs. Elucidating MHC allele identity has

been done using methods including restriction fragment length polymorphism (RFLP) analysis, cloning and sequencing, single-strand conformational polymorphism (SSCP), and direct sequencing of PCR products. However, these methods are limited by problems due to poor differentiation between alleles, failure to resolve all polymorphic sites, failure to amplify all alleles, and unreliable reproducibility (Ballingall and Tassi 2010). In species where a large proportion of known alleles are published, it is possible to screen animals with direct sequencing using robust species-specific primers. In heterozygotes, manual examination of polymorphic sites can identify known alleles, and novel alleles can be cloned and identified (Ballingall and Tassi 2010). In the case of white-tailed deer in Georgia, there is insufficient published data to rely on direct sequencing, making it necessary to identify alleles singly. To accomplish this, we will use 454 pyrosequencing following published protocols specific to the study of MHC alleles (Babik, Taberlet et al. 2009).

Previous studies of the MHC in WTD have been focused on the MHC II DRB locus, and have suggested a link between allelic diversity and a multitude of characteristics, including parasite levels and secondary sexual characteristics (Ditchkoff, Lochmiller et al. 2001; Van Den Bussche, Ross et al. 2002). For this study we analyzed the MHC Class I heavy chain, placing our focus on pathogen recognition in somatic cells, including endothelial cells. The segment we chose to analyze spans exons 2 and 3 of the class I heavy chain, which has been shown to be polymorphic in cattle. (Birch, Murphy et al. 2006).

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Table 2.1. White-tailed deer restocking history in Georgia. Adapted from the document "Deer Stocking Program in Georgia 1928-1974. Data within the published table included restocking events listed as occurring in 1978 and 1979, which have also been included in this table. Entries in the original table that described releases in parks spanning several counties were divided such that an even or nearly even number of released deer are entered for each county described. Where sexes listed in the original document were made ambiguous as regards the source population, the source information was maintained, and genders were listed as "unknown" in this table. Deer released that were collected from unknown locations were not included in this table (30 deer).

Year	Source Location	Release Site (County)	M	F	U	Total
1928	Pisgah, NC	Fannin	0	0	4	4
1928	Pisgah, NC	Fannin	0	0	8	8
1928	Pisgah, NC	Lumpkin	0	0	8	8
1928	Pisgah, NC	Union	0	0	8	8
1936	North Carolina	Habersham	0	0	6	6
1936	Pisgah, NC	Lumpkin	0	0	8	8
1936	North Carolina	Rabun	0	0	6	6
1936	Pisgah, NC	White	0	0	10	10
1938	Pisgah, NC	Gilmer	0	0	6	6
1938	Pisgah, NC	Murray	0	0	5	5
1944	Blackbeard Island	Jasper	0	0	7	7
1944	Kentucky	Jasper	0	0	11	11
1944	Wisconsin	Jasper	0	0	30	30
1944	Blackbeard Island	Jones	0	0	7	7
1944	Kentucky	Jones	0	0	11	11
1944	Wisconsin	Jones	0	0	30	30
1944	Blackbeard Island	Putnam	0	0	7	7
1944	Kentucky	Putnam	0	0	10	10
1944	Wisconsin	Putnam	0	0	30	30
1944	Blackbeard Island	Ware	0	0	30	30
1945	Wisconsin	Bartow	0	0	25	25
1947	Kentucky	Jasper	0	2	0	2
1947	Kentucky	Jones	0	1	0	1
1948	Texas	Dade	0	0	44	44

1948	Texas	Paulding	0	0	45	45
1948	Texas	Pickens	0	0	45	45
1950	Blue Ridge WMA	Banks	0	0	8	8
1950	Texas	Chamdler	0	0	40	40
1950	Blue Ridge WMA	Habersham	0	0	8	8
1950	Blackbeard Island	Jasper	0	0	15	15
1950	Blackbeard Island	Jones	0	0	15	15
1950	Blackbeard Island	Putnam	0	0	15	15
1950	Blue Ridge WMA	Stephens	0	0	8	8
1951	Blue Ridge WMA	Banks	0	0	79	79
1951	Texas	Chattahoochee	0	0	77	77
1951	Texas	McDuffie	0	0	14	14
1951	Texas	Wilkes	0	0	14	14
1952	Blackbeard Island	McDuffie	0	0	22	22
1952	Blue Ridge WMA	McDuffie	0	0	1	1
1953	Blackbeard Island	McDuffie	0	0	7	7
1955	Ossabaw Island	Rabun	0	0	55	55
1956	Ossabaw Island	Rabun	0	0	24	24
1956	Piedmont NWR	Rabun	0	0	4	4
1957	Ossabaw Island	Cherokee	0	0	27	27
1957	Texas	Cherokee	0	0	24	24
1957	Blackbeard Island	Gordon	0	0	2	2
1957	Ossabaw Island	Gordon	0	0	47	47
1957	Blackbeard Island	Greene	0	0	7	7
1957	Ossabaw Island	Greene	0	0	60	60
1957	Blackbeard Island	McIntosh	1	1	0	2
1958	Ossabaw Island	Cherokee	0	0	15	15
1958	St. Catherine's Island	Cherokee	5	14	0	19
1958	Ossabaw Island	Clinch	32	30	0	62
1958	Ossabaw Island	Clinch	4	8	0	12
1958	St. Catherine's Island	Clinch	5	8	4	17
1958	St. Catherine's Island	Clinch	2	2	6	10
1958	Ossabaw Island	Echols	1	5	0	6
1958	St. Catherine's Island	Echols	1	3	5	9
1958	St. Catherine's Island	Echols	0	3	0	3
1958	Ossabaw Island	Floyd	0	0	9	9
1958	Ossabaw Island	Gordon	0	0	9	9
1958	Ossabaw Island	Gordon	18	11	0	29
1958	St. Catherine's Island	Lanier	1	3	5	9
1958	Ossabaw Island	Talbot	29	31	0	60
1958	Ossabaw Island	Walker	0	0	9	9
1958	Ossabaw Island	Whitfield	0	0	8	8
1959	St. Catherine's Island	Cherokee	15	18	0	33

1959	St. Catherine's Island	Clinch	0	0	50	50
1959	St. Catherine's Island	Clinch	0	0	34	34
1959	Ossabaw Island	Harris	0	0	17	17
1959	Ossabaw Island	Talbot	0	0	16	16
1960	Maryland	Clarke	0	0	9	9
1960	Ossabaw Island	Clarke	0	0	12	12
1960	Ossabaw Island	Clarke	1	0	0	1
1960	Piedmont NWR	Clarke	0	0	6	6
1960	Texas	Jackson	0	0	24	24
1960	Piedmont NWR	Talbot	0	3	0	3
1961	Texas	Union	0	0	52	52
1961	Blackbeard Island	Ware	9	5	0	14
1962	Wisconsin	Appling	0	0	18	18
1962	Wisconsin	Brooks	0	0	10	10
1962	Texas	Cherokee	0	0	17	17
1962	Texas	Clarke	0	0	25	25
1962	Wisconsin	Crawford	0	0	23	23
1962	Wisconsin	Dodge	0	0	9	9
1962	Wisconsin	Dooly	0	0	10	10
1962	Wisconsin	Douglas	0	0	19	19
1962	Texas	Elbert	0	0	22	22
1962	Texas	Forsyth	0	0	32	32
1962	Texas	Gordon	0	0	25	25
1962	Texas	Gwinett	0	0	22	22
1962	Texas	Hall	0	0	25	25
1962	Wisconsin	Harris	0	0	20	20
1962	Wisconsin	Heard	0	0	22	22
1962	Wisconsin	Houston	0	0	22	22
1962	Wisconsin	Jeff Davis	0	0	17	17
1962	Wisconsin	Johnson	0	0	10	10
1962	Wisconsin	Laurens	0	0	19	19
1962	Wisconsin	Lowndes	0	0	8	8
1962	Wisconsin	Macon	0	0	19	19
1962	Texas	Ogelthorpe	0	0	23	23
1962	Texas	Rabun	0	0	42	42
1962	Wisconsin	Sumter	0	0	10	10
1962	Wisconsin	Taylor	0	0	22	22
1962	Wisconsin	Telfair	0	0	8	8
1962	Texas	Towns	0	0	30	30
1962	Wisconsin	Troup	0	0	22	22
1962	Wisconsin	Upson	0	0	20	20
1962	Piedmont NWR	Ware	11	18	0	29
1962	Wisconsin	Wheeler	4	15	0	19

1962	Wisconsin	Wilcox	0	0	20	20
1963	Texas	Banks	0	0	24	24
1963	Texas	Carroll	0	0	12	12
1963	Univ of Georgia	Clarke	2	4	0	6
1963	Texas	Douglas	0	0	12	12
1963	Texas	Elbert	0	0	25	25
1963	Texas	Floyd	0	0	12	12
1963	Texas	Gilmer	0	0	26	26
1963	Texas	Gordon	0	0	22	22
1963	Texas	Gwinett	0	0	24	24
1963	Texas	Hall	0	0	49	49
1963	Piedmont NWR	Jeff Davis	4	12	0	16
1963	Texas	Ogelthorpe	0	0	24	24
1963	Texas	Pickens	0	0	23	23
1963	Texas	Polk	0	0	11	11
1963	Texas	Wilkes	0	0	24	24
1964	Piedmont NWR	Dooly	0	8	0	8
1964	Jekyl Island	Dougherty	18	24	0	42
1964	Piedmont NWR	Dougherty	1	1	0	2
1964	Jekyl Island	Houston	2	4	0	6
1964	Jekyl Island	Laurens	4	4	0	8
1964	Jekyl Island	Laurens	0	0	31	31
1964	N. Carolina	Lee	0	0	11	11
1964	Piedmont NWR	Lowndes	2	14	0	16
1964	Jekyl Island	Pierce	4	4	0	8
1964	Jekyl Island	Stephens	2	7	0	9
1964	Jekyl Island	Tift	0	0	31	31
1964	Jekyl Island	Tift	2	8	0	10
1964	Jekyl Island	Wilcox	0	0	31	31
1964	Jekyl Island	Wilcox	3	3	0	6
1965	Texas	Bartow	0	0	12	12
1965	Texas	Carroll	0	0	10	10
1965	Texas	Chattoga	0	0	10	10
1965	Okeefenokee Swamp	Coffee	4	0	0	4
1965	Ossabaw Island	Coffee	9	3	0	12
1965	Jekyl Island	Dooly	5	2	0	7
1965	Quantico Marine Base, VA	Dougherty	0	1	0	1
1965	Jekyl Island	Emanuel	3	5	0	8
1965	Okeefenokee Swamp	Jenkins	1	3	0	4
1965	Jekyl Island	Laurens	3	5	0	8
1965	Texas	Pike	0	0	16	16
1965	Jekyl Island	Troup	3	7	10	20

1965	Texas	Walker	0	0	15	15
1965	Jekyl Island	Wheeler	4	3	0	7
1965	Texas	Whitfield	0	0	12	12
1968	Berry College	Fannin	0	0	1	1
1968	Dade Co. (Lookout Mt.)	Fannin	0	0	1	1
1968	Franklin, Co	Fannin	0	0	1	1
1968	Glynn Co.	Fannin	1	4	0	5
1968	Glynn Co.	Fannin	0	0	5	5
1968	Clark Hill WMA	Gilmer	4	8	0	12
1968	Blairsville, GA	Murray	1	0	0	1
1969	Berry College	Gilmer	10	12	17	39
1969	Clark Hill WMA	Gilmer	3	3	0	6
1969	Clark Hill WMA	Treutlen	2	5	0	7
1970	Berry College	Murray	0	0	33	33
1970	Berry College	Murray	0	3	0	3
1970	Polk Co.	Murray	0	0	2	2
1970	Clark Hill WMA	Treutlen	0	6	0	6
1971	Berry College	Appling	1	2	0	3
1971	Berry College	Catoosa	0	4	8	12
1971	Berry College	Dodge	6	5	0	11
1971	Berry College	Gilmer	3	4	0	7
1971	Berry College	Jenkins	0	1	0	1
1971	Berry College	Murray	0	0	19	19
1972	Sapelo Island	Appling	0	0	1	1
1972	Berry College	Bulloch	2	2	0	4
1972	Gordon Co.	Catoosa	0	1	0	1
1972	Candler Co.	Coffee	0	0	6	6
1972	Sapelo Island	Coffee	0	0	6	6
1972	Berry College	Coweta	0	1	0	1
1972	Berry College	Gilmer	3	28	0	31
1972	Berry College	Gilmer	0	0	25	25
1972	Walker Co.	Gilmer	0	0	1	1
1972	Berry College	Murray	0	2	0	2
1972	Berry College	Union	0	1	0	1
1973	Berry College	Fannin	0	0	14	14
1973	Berry College	Gilmer	0	0	10	10
1973	Berry College	Gordon	0	0	11	11
1973	Berry College	Pickens	0	0	10	10
1973	Sapelo Island	Pierce	0	0	8	8
1973	Berry College	Union	0	0	15	15
1973	Sapelo Island	Wayne	0	0	8	8
1974	Berry College	Cherokee	6	16	0	22
1974	Berry College	Cherokee	6	16	0	22

1974	Sapelo Island	Dodge	11	4	0	15
1974	Berry College	Gilmer	0	0	14	14
1974	Berry College	Gordon	0	0	14	14
1974	Sapelo Island	McIntosh	1	2	0	3
1974	Berry College	Pickens	0	0	14	14
1974	Paradise Parke	Pierce	0	0	10	10
1974	Paradise Parke	Wayne	0	0	11	11
1974	Berry College	Whitfield	2	14	0	16
1975	Berry College	Catoosa	20	52	0	72
1975	Berry College	Dade	15	20	0	35
1978	Berry College	Fannin	3	1	0	4
1979	Berry College	Fannin	0	0	2	2
1979	Clark Hill WMA	Fannin	0	0	2	2

CHAPTER 3

POPULATION GENETIC STRUCTURE IN WHITE-TAILED DEER AND ITS POTENTIAL INFLUENCE ON HEMORRHAGIC DISEASE MORTALITY[1]

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ABSTRACT

Hemorrhagic disease (HD) is the most significant infectious disease of white-tailed deer (WTD) in the southeastern USA. Severe HD resulting in mortality is most often associated with northern latitudes, and results from experimental infection studies provide evidence for the role of innate host factors in HD resistance. In Georgia (GA), a consistent geographic pattern of mortality has prompted speculation regarding the potential role of spatial variation in host innate factors introduced to the state during extensive restocking efforts to reestablish the species following near extirpation in the early 20th century. This study estimated the population genetic structure of white-tailed deer in GA using mitochondrial and microsatellite markers, compared extant deer from GA and Wisconsin (WI) to identify residual genetics from source populations, and evaluated Georgia deer for disparities in allelic diversity at the major-histocompatability (MHC) class I heavy chain locus. Metapopulations across the state demonstrate varying degrees of genetic isolation, and two isolated populations (Blue Ridge Mountains, barrier islands; $p<0.05$ for mtDNA) are associated geographically with increased HD mortality reports. Of 16 mitochondrial D-loop control haplotypes identified in 21 Wisconsin WTD, 7 (43.8%) were identical to haplotypes found in GA WTD, and were associated spatially with counties where WI deer were introduced in the mid 1900s. No significant differences were found in allelic diversity at the MHC Class I heavy chain locus across sampled locations. Our findings suggest fine-scale genetic structure in GA WTD, influenced by introduction history, with isolated metapopulations corresponding geographically with areas of increased HD mortality. However, host-pathogen dynamics are complex, and host innate factors represent only a portion of potential variables influencing the geographic distribution of HD mortality in GA.

Key words: white-tailed deer, population genetics, hemorrhagic disease, bluetongue, Georgia, Wisconsin

INTRODUCTION

Hemorrhagic disease (HD) is the most significant infectious cause of mortality in white-tailed deer (*Odocoileus* virginianus, WTD) in the southeastern United States, including the state of Georgia (GA) (Gaydos, Crum et al. 2004). This clinical syndrome is caused by infection with viruses of the closely related bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) serogroups, with disease presentation varying from inapparent infection to acute death. Sequelae in survivors include interrupted hoof walls and rumen scarring, which may result in loss of body condition (Murphy, Howerth et al. 2005). Distribution of HD epizootics is geographically non-uniform, with infrequent but clinically severe outbreaks occurring in the northern latitudes, and mild to absent clinical disease in the southwest (Davidson and Doster 1997). Serological surveillance suggests frequent exposure to virus in southern latitudes, where virus and host coexist in areas of apparent enzootic stability (Stallknecht, Luttrell et al. 1996). Experimental EHDV infection of naïve northern (Pennsylvania, *O. v. borealis*) and southwestern (Texas, *O.v.texanus*) deer found significant differences in clinical disease and mortality between these subspecies, suggesting an innate component of immunity to HD (Gaydos, Davidson et al. 2002). The spatial pattern of HD mortality in GA has been historically consistent over 29 years of surveillance at the Southeastern Cooperative Wildlife Disease Study (SCWDS, Figure 3.1). It is thought that such variation in disease prevalence may be attributed to a complexity of factors, including the pathogenicity of viruses present, the abundance and competency of local vectors (*Culicoides spp.*), herd immunity, and host genetics (Gaydos, Crum et al. 2004). The genetics of southeastern WTD have been shown to be disproportionately heterogeneous spatially, likely due to extensive restocking efforts initiated in the early 20th century (Purdue, Smith et al. 2000; DeYoung, Demarais et al. 2003). If we are to surmise that geographic disparities in HD mortality

in GA are influenced by host genetic factors, we assume that these areas are genetically distinct, suggesting fine-scale population structuring. While this may seem unlikely given the size and vagility of this species, reports in landscape ecology and genetics have described reduction in gene flow in similar species by relatively minor barriers (Coulon, Guillot et al. 2006). Additionally, following the near extirpation of WTD from the state by 1900, introduced deer may have had profound effects on contemporary populations, resulting in greater spatial heterogeneity than would be observed in native deer populations. Susceptibility to HD in these source populations could conceivably influence current disease dynamics, especially in areas where viral exposure is sporadic. During the WTD restocking program in Georgia (1928-1979), hundreds of deer were introduced from Wisconsin, where EHD and BT viruses have not been shown to be endemic (Wilhelm and Trainer 1966). This state is also within the reported range of the *O. v. borealis* subspecies analyzed in the innate resistance infection studies described above (Gaydos, Davidson et al. 2002; Rue 2004). In addition to population structure, intra-population genetic diversity may play a role in disease dynamics. It has been suggested that diversity at major histocompatibility complex (MHC) loci may be associated with disease resistance, though results have been varied (Ditchkoff, Lochmiller et al. 2001).

We hypothesize that WTD population structure in GA is sufficient to account for spatial disparities in HD mortality, with areas of relatively high mortality being genetically distinct from areas inhabited by apparently resistant herds. We will characterize GA WTD population genetics by evaluating a mitochondrial marker (D-Loop control region, mtDNA CR) and a panel of microsatellite markers. Mitochondrial haplotypes in GA deer will also be compared to WI WTD to identify genetic influences from introduced deer. The major histocompatibility class I heavy

chain locus will be evaluated for each sampled population to determine if HD mortality may be correlated with reduced MHC haplotype diversity.

MATERIALS AND METHODS

Calculating HD mortality indices

Reports of HD in WTD were compiled from records maintained at the Southeastern Cooperative Wildlife Disease Study from 1980-2009, and an index was created to summarize and spatially represent mortality and morbidity data by county across GA.

Estimating regions affected by introduced WTD

White-tailed deer were introduced into GA from several disparate sites, including Texas (TX), Appalachia/Eastern US (NC, MD, KY; these have been clumped as numbers from each are small), and Wisconsin (WI). While TX represents an area of apparent enzootic stability between virus and host, WI and Eastern deer have historically experienced sporadic HD epizootics. Historical records summarized and published by the Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission were screened for entries describing introductions of deer from out of state, including WI, TX, and Eastern deer (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975). In order to map county estimates of deer introduced up to 50 years apart, the data was normalized to account for temporal disparities. The equation for density-independent population growth was used to equalize introduced numbers to contemporary levels, assuming founder stock survival ($N=N_o e^{rt}$, where N=population size, N_o=starting population, e=the natural log base, r=a constant rate of growth, and t=time). The

number of released deer was considered the starting population, *t* was the number of generations (assumed 2 years for WTD) between the release year and 2011, and 0.01 was arbitrarily chosen as the constant rate of growth (Lancia, Rosenberry et al. 2000). Normalized numbers of released deer from the 1928-1979 restocking program were mapped by county using the dot density function in ARCGIS 10 (www.esri.com), and compared to the HD mortality index.

Specimens examined

Skeletal muscle samples were collected from hunter-harvested white-tailed deer at 15 wildlife management areas (WMAs) and 1 Georgia State Park (SP) during the hunting seasons of 2005 and 2006. The Georgia Department of Natural Resources (DNR) provided access to hunter check-stations at these locations, which were selected to optimize coverage of the state and include areas with both high and low levels of reported HD mortality. Lymph nodes from WTD collected in WI were provided by the Wisconsin Department of Natural Resources and further processed and analyzed at SCWDS. Samples were stored frozen (-20 °C) prior to further processing.

Laboratory methods

Extraction of DNA was performed using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. The mitochondrial D-Loop control region was amplified with oligonucleotide primers designed for WTD sequence. Sequence of GA WTD mtDNA CR was amplified using the primers L15926 and H16498 (Pease, Freedman et al. 2009). Similar sequences provided by a nucleotide BLAST query (blast.ncbi.nlm.nih.gov) were compared for conserved regions in published WTD sequences. Primers for GA WTD were selected by eye and optimized with NetPrimer (www.premierbiosoft.com/netprimer). Primers DL102809F (5'-

CCCTAAGACTCAAGGAAGAAG-3') and DL102809R (5'-TATGGGGATGCTCAAGATGC-3') amplify an 847-bp segment of the WTD mtDNA CR. Samples were amplified using GoTaq® Flexi DNA polymerase and the following the thermocycler protocol: 1 min denaturation (94 °C) followed by 30 cycles of 30 s denaturation (94 °C), 30 s annealing (59 °C), and 30 s elongation (72 °C), with a final 2 minute elongation step (72 °C). Products were confirmed visually with agar gel electrophoresis prior to enzyme purification with the following protocol: 5µL of PCR product is incubated in a mix of 1µL Antarctic phosphatase, 1µL exonuclease I, 1uL Antarctic phosphatase buffer, and 2µL molecular biology grade water at 37 °C for 15 min and then in 80 °C for 15 min. Samples were then submitted to University of Georgia's Georgia Genomics Facility for Sanger sequencing using both the DL102809F and DL102809R primers on an ABI 3730xL capillary sequencer. Sequences were visualized and edited using Chromas Lite (www.technelysium.com.au). Sequences were aligned and trimmed in CLC Sequence Viewer (alignment parameters: gap open cost =20, gap extension cost=0, end gap cost=free, www.clcbio.com).

Twenty-one microsatellites published for use in WTD were screened for use in multilocus genotyping with universal fluorescently labeled primers (Anderson, Honeycutt et al. 2002). An engineered sequence was adhered to the 5' terminus of one primer from each pair (CAG tag, 5'-CAGTCGGGCGTCATCA-3') to enable binding of a third fluorescently labeled (NED or 6-FAM) universal primer in the PCR (Peters, Ovenden et al. 2009). Loci were amplified with a touchdown PCR thermocycler program as follows: 2 min denaturation at 94°C followed by cycles of 30 s at 94°C, 30 s at a variable annealing temperature, and 30 s at 72°C. The annealing temperatures ranged from 58°C to 48°C and dropped 0.5°C every second cycle, with a final 15 cycles at 48°C. A final extension was performed at 72°C for 2 mins. Amplified PCR products

were combined with a standard ladder (GGFROX500, provided by GGF) and submitted to the Georgia Genomics Facility (GGF) for fragment analysis on an ABI 3730xL capillary sequencer. Alleles were scored using GeneMapper software (Applied Biosystems). In order to increase statistical power and minimize potential genotyping error, allele sizes were assigned based on the assumptions of the step-wise model.

To evaluate MHC diversity in GA WTD, a portion of the MHC I heavy chain locus was amplified using cattle MHC class I primers Bov 7 (exon 2) and Bov 11 (exon 3) (Birch, Murphy et al. 2006). Amplified sequences from GA WTD were aligned and WTD-specific primers were designed and analyzed using NetPrimer (WTD-MHCI F 5'-GTTTTTCCGAATGAGCCT-3', WTD-MHCI 5'-CTTCTTTGACCGCCTCTGAC-3', Table 3.1). The highest quality PCR product of ideal size for 454 pyrosequencing was amplified with hemi nested PCR; the primary reaction using primers Bov 7 and Bov 11, and the secondary reaction primed with Bov 7 and WTD MHCI F. A set of each of the 2° primers were labeled with 10 different MIDI tags to correspond with the 10 sampled locations (Table 3.1). Extracted DNA from ten individuals from each of 10 sampling sites was amplified using the touchdown PCR protocol described above, quantified spectrophotometrically (NanoDrop, Thermo Scientific), and pooled in equal proportions prior to submission to the Georgia Genomics Facility for 454 pyrosequencing.

Data analysis

Mitochondrial DNA

Standard diversity parameters including population allele frequencies, haplotype diversity and nucleotide diversity were calculated using ARLEQUIN v.3.5.1.2 (Excoffier, Laval et al. 2005). A neighbor-joining phylogenetic tree was constructed (1000 bootstrap iterations under the

Maximum composite Likelihood model) in MEGA v.4.0 (Tamura, Dudley et al. 2007), visualized using FIGTREE v.1.3.1 (tree.bio.ed.ac.uk/software/figtree) and spatially represented in 3-dimensions using GEOPHYLOBUILDER v1.0 (Kidd and Liu 2008) and ArcGIS 10 (www.esri.com). A median-joining network analysis was performed using NETWORK v.4.6 (www.fluxus-engineering.com) to compare haplotypes across GA and WI, and identify haplogroups. Pairwise F_{ST} values were calculated between sampling sites using the Tajima and Nei correction in ARLEQUIN, allowing for unequal nucleotide frequencies. Also in ARLEQUIN, multiple AMOVA calculations were performed using user-determined *a priori* grouping scenarios suggested by geographic proximity, haplogroup distributions and the results of pairwise F_{ST} calculations (10000 permutations for significance). Proposed population structure scenarios were compared to determine the least amount of structure that would maximize among-group variability (maximizing the sum of squares) while maintaining non-significant within-group variability ($p<0.05$). Sequences obtained from WI WTD were compared to GA WTD in ARLEQUIN to identify shared alleles, and in NETWORK, to identify haplogroups associated with WI WTD.

Microsatellite DNA

Microsatellite loci were analyzed using MICRO-CHECKER v2.2.3 for the presence of stutter peaks, null alleles, and large allele dropout (Van Oosterhout 2004). Pairwise and global tests for Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP v.4.0 (Rousset 2008), with 20 batches of 5000 Markov chain iterations. Pairwise tests for linkage disequilibrium were performed in ARLEQUIN, with 10000 permutations. Pairwise R_{ST} values, allelic diversity, overall allele size range, and observed and expected heterozygosities by population were calculated using GENALEX v.6.1 (Peakall and Smouse 2006). Allelic richness (A_C) was

calculated for each sampled site, based on a minimum of 10 sampled individuals using FSTAT v.2.9.3.2. (Goudet 1995). Putative population structure in GA WTD was analyzed using microsatellite genotypes and STRUCTURE v2.3.3. (Pritchard and Wen 2003). The length of burn-in period was set at 10,000 and each iteration ran 10,000 Monte Carlo Markov chain (MCMC) repetitions, determined to be statistically sound by Evanno et al (Evanno, Regnaut et al. 2005). Analyses were performed under the admixture model, which allows for individuals to be of mixed ancestry, in parallel with the correlated allele frequencies model (optimal in cases of subtle genetic structure (Falush, Stephens et al. 2003)). The degree of admixture alpha values were allowed to be inferred from the data. All other options were set to the default values or those suggested by program documentation.

Major histocompatability complex

Sequence fragments identified by 454 pyrosequencing of WTD MHC I were assembled into 99% sequence identity bins using CAP3 (99% SIR, 300bp overlap). Then, using the methods of Babik et al. (Babik, Taberlet et al. 2009), we identified putative alleles as sequences that were held in their respective populations at a frequency of greater than 1%. For each allele, frequency of occurrence in each population were subjected to neighbor-joining within Phylip. Allelic diversity by sampling site was compared to identify differences in MHC haplotypic diversity. The data was examined for private alleles or alleles present in only areas of either high or low HD mortality.

RESULTS

Hemorrhagic disease prevalence data indices

Maps were created from compiled HD reports across the state and an index was created to summarize WTD HD mortality levels by county, which was visualized colorimetrically in ArcGIS 10. Mortality is reported most often in the northeastern mountains and in the southeastern piedmont (Figure 3.1, see Fig 3.8b for GA physiogeographic regions), and morbidity reports (primarily observations of interruptions of the hoof wall) occur most often in the southwest and upper-coastal plain (Figure 3.1b). Sampling sites were selected to optimize coverage of these various areas (Figure 3.1).

Mapping WTD introductions by county

Comparing restocking history to prevalence of clinical HD found that the two areas of highest HD index in the central Piedmont and Blue Ridge mountains correlate directly with deer introduced from Appalachia/Eastern US (NC, KY, MD), areas associated with HD mortality (Figure 3.2a). Counties where WI deer were introduced do not correlate overall with areas of HD mortality, though the central Piedmont HD hotspot was also repopulated with WI deer. The remaining counties where WI WTD were released were in the western to central coastal plain, western piedmont, and southern valley and ridge regions, which are characterized by relatively higher incidence of HD morbidity reports (Figure 3.2b). Introductions of Texas deer were not correlated overall with areas characterized by consistent mild to inapparent disease, which is true for most of their native state. Texas WTD were introduced into a wide range of areas of the state, with greatest concentration across the northern and eastern Piedmont and the Blue Ridge mountains (Figure 3.2e,f).

mtDNA sequence analysis and genetic diversity

Following alignment and trimming, the analyzed portion of the mtDNA CR was 790bp, with 752 bases containing less than 10% missing data (insertions were present in a subset of individuals). At 16 total sampling sites, from 5 (West Point WMA) to 19 (Berry College WMA) individuals were sequenced and included in analysis (mean=11.2, median=11.5, mode=12). Across all 179 deer sequenced in Georgia, a total of 200 nucleotide loci were polymorphic. The number of polymorphic nucleotides per sampling site ranged from 3 (Ossabaw Island WMA) to 131 (Riverbend WMA), with a mean of 51 polymorphic loci per sampling site. The number of distinct haplotypes identified per site ranged from 2 (Ossabaw Island WMA) to 7 (Blanton Creek WMA and Cedar Creek WMA). Haplotype diversity values ranged from 0.2 (Ossabaw Island WMA) to 0.89 (Cedar Creek WMA), and nucleotide diversity calculations varied from 0.001 (Ossabaw Island WMA) to 0.051 (Lake Russell WMA, Table 3.2). The neighbor-joining (N-J) phylogenetic tree constructed in MEGA v.4 identified 2 distinct clades, Clade I includes all barrier island deer and most deer in the Blue Ridge mountains, while Clade II includes most of the deer in the GA coastal plain and western Piedmont (Figure 3.3a). A 3D visualization of the N-J tree depicts this differentiation in space, with Clade II position at greater elevation than Clade I (Figure 3.3b). Computed pairwise F_{ST} values demonstrated significant differences between 98 site pairs (Table 3.3). Of the multiple AMOVA calculations performed, the grouping scenario demonstrating the least genetic structure while maximizing among group variance and maintaining non-significant within-group variance ($p<0.05$) was found to be grouping J, which combined deer in the Georgia coastal plain (DM and RB, though not CH), deer in the western and southern piedmont (JK, BC, BL, WP, BF, CC) and northern and eastern piedmont (DF, FY, CL), while keeping all over sampling sites distinct (Table 3.4, Figure 3.4). The median-joining

network depicts two large clades of haplotypes, with an additional single haplotype that belongs to neither (a private haplotype of Lake Russell WMA). The clades can be further subdivided, for a total of 10 haplogroups (identified and color coded, Figure 3.5a). Line lengths in the network are proportional to the number of estimated mutation events between haplotypes, with the shortest lines representing a single nucleotide change. Geographic haplotype distribution is represented by color coded pie charts identifying haplotypes identified at each sampling site. Each distinct haplotype is represented by a wedge, while colors correspond to haplogroup affiliations (Figure 3.5b).

Estimated areas influenced by introduced WI WTD

Of the 21 sequenced WI WTD, 16 haplotypes were identified, 7 of which were identical to haplotypes found in GA WTD. Pie charts depicting haplotype frequencies for each sampling site in GA demonstrate the proportion of shared WI haplotypes per site (Figure 3.6a). Mapped extrapolated WI WTD descendants suggests that contemporary deer in the western and central coastal plain, and western piedmont are most likely to be have been founded at least in part by WI deer, assuming founder stock survival. Of 16 total sites sampled in Georgia, 9 fall in this range, 7 of which share haplotypes with WI at a frequency of at least 25% (Figure 3.5a). No other sampled sites had haplotypes in common with the sampled WI deer. The median-joining network constructed for GA WTD included WI sequences. All haplotypes amplified from the 21 sequenced WI WTD belong to haplogroups 1 and 4 (with a single outlier found only in 1 WI sample, Figure 3.6b).

Microsatellite genotyping and genetic diversity

Of the 21 screened microsatellites, 10 were readily amplified using the CAG-tag universal primer system and standardized thermocylcer protocol. Of these, 6 were tetranucleotide repeats (N, Q, K, O, R, D), and 4 were dinucleotide repeats (BM6438, BM848, INRA011, ETH152). Following analysis of genotyping data using MICROCHECKER, 1 microsatellite locus (ETH152) was determined to have consistent heterozygote deficiency likely due to repeated genotyping error from stutter peaks, and was omitted from further analysis. Over all 275 deer genotyped from GA, 8 of the 9 remaining loci demonstrated significant heterozygote deficiency with no evidence of genotyping error due to stutter peaks or large allele dropout. Global tests for HWE found significant heterozygote deficiency in 6 of 9 loci, in 7 of 11 sampling sites (*U*-test, $p<0.05$). No significant heterozygote excess was found across any loci or populations. Pairwise tests rejected HWE in 31 of 99 tests, with significant heterozygote deficiency. Of 396 pairwise tests of loci within populations, 37 were significantly linked ($p<0.05$). All 9 loci were polymorphic, with 115 alleles genotyped across all 9 loci (Figure 3.7). Eleven sampling sites were genotyped, analyzing between 10-30 animals (mean=27.8, median=28, mode=30) per location. The mean number of alleles per site varied from 5.67 (Fort Yargo SP) to 8.33 (Riverbend WMA). Sample size disparity was corrected by calculating allelic richness, which varied from 5.03 (Lake Russell WMA) to 6.74 (Ossabaw Island WMA). The range of allele size in each group ranged from 7.11 (Fort Yargo SP) to 10.22 (Joe Kurz WMA). Significant pairwise R_{ST} levels were calculated between 14 pairs of sampling sites (Table 3.3), suggesting that these sites are distinct based on allele frequencies and size differences following the step-wise model incorporated into R_{ST} calculations. For cluster assignment tests in STRUCTURE, 5 iterations were run for proposed population number (K) 1-12. Values of *Ln Pr(X|K)* peaked at both K=5

and 10, though values above K=5 had increased standard error (Figure 3.8). The most clearly geographically assigned clusters are in the ridge and valley (Berry College WMA), western coastal plain (Chickasawhatchee WMA), Blue Ridge Mountains (Cohutta WMA), and barrier islands (Ossabaw Island WMA), all demonstrating greater than 60% of each sample population to a single private cluster, with additional assignments to each cluster near the levels associated with random chance (20% per cluster, K=5; Table 3.5a). Assignment proportions to k=10 clusters demonstrated clustering similar to assignments under k=5 calculations. The above listed sampling sites (BE, CH, and OI) assigned to private clusters (with no other sample sites assigned at proportions above those expected due to random sampling, 10% for k=10) at proportions greater than 50%. Cohutta WMA assigned to a cluster shared by greater than 20% of Dawson Forest WMA samples. Dixon Memorial WMA (southeastern coastal plain) was assigned to 2 private clusters, one with a sample proportion greater than 40%, and another with greater than 20% assignment (Table 3.5b). Clustering data for both K values were visualized with DISTRUCT v1.1 (Rosenberg 2004), depicting high cluster fidelity for both Ossabaw Island and Berry College WMA sampling sites, and admixture in the remaining sites with colorimetric suggestion of additional structure consistent with data listed above (Figure 3.9).

MHC I genotyping and diversity

Hemi-nested PCR resulted in products of approximately 490bp, tagged at both ends with a MIDI tag specific to sampling location. Pyrosequencing of 10,000 reads of products amplified from 100 sampled individuals from 10 locations resulted in 27 distinct alleles. Allelic diversity ranged from 9 alleles (Ossabaw Island WMA) to 20 alleles (BF Grant WMA). There were 2 private alleles, a8 (BF Grant WMA) and a23 (Dixon Memorial WMA). No alleles were associated only with areas of high or low HD mortality (Table 3.5). An unrooted phylogenetic tree of sampling

sites demonstrated no consistent correlation with geographic proximity or HD mortality levels (Figure 3.10).

DISCUSSION

The primary objective of this study was to characterize the genetic structure of GA WTD with the aim of elucidating potential correlations with HD mortality levels. Compiling and mapping extrapolated numbers of descendants from introduced deer clarified areas potentially influenced by foundation stock from widely distant regions. When comparing the source locations of introduced foundation stock and HD mortality data, there is no consistent correlation of elevated HD mortality and susceptible source populations. However, a single source of deer from an HD epizootic area (Eastern US) correlates with the two areas of highest relative HD mortality (central Piedmont and Blue Ridge mountains, Figure 3.2c), and areas restocked with WI deer are associated with either higher levels of HD mortality (central Piedmont, Figure 3.2a) or morbidity (western to central coastal plain, Fig 3.2b). Assuming survival of founder stock, these data suggest introductions may have influenced modern trends in HD infection outcome. Comparisons of mtDNA in GA and WI WTD found identical haplotypes shared between the two states. Distribution of these sequences in GA corresponds spatially to sites restocked with Wisconsin deer, suggesting residual genetics from foundation stock and providing evidence contrary to suggestions that these introduced deer did not survive (DeYoung, Demarais et al. 2003).

Evaluation of MHC Class I allelic diversity demonstrated only mild variation in diversity levels across the state, and little geographic correlation with allele frequencies at sampling sites.

These findings suggest that disparities in MHC Class I diversity do not account for the geographic distribution of HD mortality in Georgia.

Estimation of population genetic structure across Georgia varied between analyses of mtDNA and microsatellites, which is to be expected in a polygynous species like white-tailed deer, which are characterized by sex-biased dispersal (DeYoung, Demarais et al. 2009; Perez-Espona, Perez-Barberia et al. 2010). Given the relative philopatry of does, it is unsurprising that the population structure suggested by examination of a matrilinear marker (mtDNA CR) would result an estimation of greater genetic structure than nuclear markers (microsatellites) (Purdue, Smith et al. 2000). Wright's F_{ST} and AMOVA calculations (Tables 3.3 and 3.4) defined 8 clusters of deer in the state from samples originating from 16 sites across the state (Figure 3.3). These subdivisions identify distinct populations in the northern Blue Ridge Mountains (Cohutta WMA) and valley and ridge region (Berry College WMA), as well at the Georgia-South Carolina border in the far eastern piedmont (Lake Russell WMA) and barrier islands (Ossabaw Island WMA). The coastal plain was divided between west and east, and the piedmont between southwest and northeast. Calculated HD mortality indices demonstrate elevated levels in the central piedmont, Blue Ridge Mountains, and to a lesser extent, barrier islands. While the Blue Ridge Mountains and barrier islands are estimated to be distinct populations, the central piedmont is not significantly different from the western piedmont, which does not share the level of HD mortality (though western piedmont does have elevated incidence, Figure 3.1a). Analysis of a subset of these sampling sites using microsatellite markers suggests less genetic structure across the state based on *a priori* population definitions (sampling sites, R_{ST} values, Table 3.3). However, allowing for clustering without prior population designations using Bayesian assignment algorithms identified 5 probable clusters, with a second peak in posterior

probabilities at k=10 (Figure 3.9). Each of these clustering scenarios places more than 50% of samples in the Chickasawhatchee WMA (western coastal plain), Berry College WMA (ridge and valley), and Ossabaw Island WMA (barrier islands) sites to private clusters (with no other sites assigned with proportions greater than would be expected under random sampling). With 5 inferred clusters, Cohutta WMA (Blue Ridge Mountains) is assigned to a private cluster, though at k=10 inferred clusters, its members share a cluster with Dawson Forest WMA (Blue Ridge Mountains). With 10 inferred clusters, Lake Russell WMA (eastern piedmont) is also assigned to a private cluster, with a proportion of greater than 20% of its members assigned. These assignments concur with population divisions estimated by analysis of mtDNA CR sequences (Figure 3.2). These clusters concur with the estimated population structure identified by mtDNA analysis with additional evidence of admixture. These results suggest that while there is gene flow between Georgia white-tailed deer populations, there are differences in the level of restriction of this flow, with resulting metapopulations. These relatively isolated populations are located in the Blue Ridge Mountains, Barrier Islands, and valley and ridge regions of the state. Deer in the western coastal plain share limited genetics with herds in the central and eastern coastal plain. Conversely, microsatellite analyses suggest that the piedmont is comprised of an admixed population, with the exception of the far eastern Georgia border. This spatial arrangement of population structure suggests sufficient heterogeneity to conceivably be a factor in phenotypic variation, including disease response, though phenotypic variation is notably unreliably inferred from genotypic variation. The phenotype of interest in this study is to the potential association between high HD mortality and distinct populations, which would provide support for host genetic factors in clinical HD outcomes. The results of these analyses suggest genetic isolation of WTD in the Blue Ridge Mountains and barrier islands, which have elevated

HD mortality reporting. However, these analyses also suggest a panmictic population across most of the piedmont of Georgia, though HD mortality levels across this area are not uniform. Host-pathogen dynamics are undeniably governed by multivariate influences, of which host genetic factors represent only a proportion. However, our findings suggest that spatial heterogeneity across Georgia demonstrates sufficient restrictions in gene flow to allow for differences in host genetic factors to remain on the list of variables potentially affecting the distribution of clinical HD.

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Table 3.1. MIDI-tagged WTD MHC I primer sequences. Hemi-nested PCR was performed using the MHC-F primers below (primer Bov 7 with 5' MIDI tag) specific to each location. Secondary reactions were primed using the MHC-F and R (primer WTD-MHC-F with 5' MIDI tag)**.** MIDI tag sequences are in bold.

Site Name	Primer Name	Primer Sequence
Berry College WMA	MHC-F-1	**ACGAGTGCGT**GTTTTTCCGAATGAGCCT
	MHC-R-1	**ACGAGTGCGT**CCCTCCAGGTAGTTCCT
BF Grant WMA	MHC-F-2	**ACGCTCGACA**GTTTTTCCGAATGAGCCT
	MHC-R-2	**ACGCTCGACA**CCCTCCAGGTAGTTCCT
Chickasawhatchee WMA	MHC-F-3	**AGACGCACTC**GTTTTTCCGAATGAGCCT
	MHC-R-3	**AGACGCACTC**CCCTCCAGGTAGTTCCT
Cohutta WMA	MHC-F-7	**CGTGTCTCTA**GTTTTTCCGAATGAGCCT
	MHC-R-7	**CGTGTCTCTA**CCCTCCAGGTAGTTCCT
Dawson Forest WMA	MHC-F-4	**AGCACTGTAG**GTTTTTCCGAATGAGCCT
	MHC-R-4	**AGCACTGTAG**CCCTCCAGGTAGTTCCT
Dixon Memorial WMA	MHC-F-8	**CTCGCGTGTC**GTTTTTCCGAATGAGCCT
	MHC-R-8	**CTCGCGTGTC**CCCTCCAGGTAGTTCCT
Joe Kurz WMA	MHC-F-5	**ATCAGACACG**GTTTTTCCGAATGAGCCT
	MHC-R-5	**ATCAGACACG**CCCTCCAGGTAGTTCCT
Lake Russell WMA	MHC-F-6	**ATATCGCGAG**GTTTTTCCGAATGAGCCT
	MHC-R-6	**ATATCGCGAG**CCCTCCAGGTAGTTCCT
Ossabaw Island WMA	MHC-F-9	**TCTCTATGCG**GTTTTTCCGAATGAGCCT
	MHC-R-9	**TCTCTATGCG**CCCTCCAGGTAGTTCCT
Riverbend WMA	MHC-F-10	**TGATACGTCT**GTTTTTCCGAATGAGCCT
	MHC-R-10	**TGATACGTCT**CCCTCCAGGTAGTTCCT

Table 3.2. Genetic diversity of Georgia white-tailed deer by sampling site. Of 16 sampling sites in Georgia, two groups combine indicated populations. Diversity indices are calculated from the mitochondrial D-Loop control region (mtDNA CR, 690bp), 9 polymorphic microsatellite loci, and a portion of the MHC II heavy chain. Total number of samples analyzed per population or group (N_T), total individuals mtDNA-sequenced (n_{mt}), number of haplotypes per site (n_h), haplotype (h) and nucleotide (π) diversities (± standard deviation, SD), total microsatellite-genotyped individuals (n_{mic}), mean alleles per locus (A), allelic richness (A_C), allele size range (r_{AS}), observed (H_O) and expected (H_E) heterozygosities (± standard deviation, SD), number of individuals MHC-sequenced (n_{MHC}) and total MHC alleles per sampling site (A).

		mtDNA CR					Microsatellite loci						MHC II	
	N_T	n_{mt}	n_h	h ± SD	n_{ps}	$\pi \pm SD$	n_{mic}	A	A_C	r_{AS} ± SD	H_O ± SD	H_E ± SD	n_{MHC}	A
Berry College WMA	28	19	3	0.37 ± 0.13	41	0.017 ± 0.009	28	6.67	5.11	9.44 ± 4.26	0.60 ± 0.17	0.68 ± 0.15	10	-
West GA Group	59	41	13	0.90 ± 0.02	74	0.024 ± 0.012	30	8.11	6.07	10.22 ± 4.09	0.62 ± 0.17	0.72 ± 0.18	10	19
Big Lazer WMA	12	12	4	0.80 ± 0.06	25	0.017 ± 0.009	-	-	-	-	-	-	-	-
Blanton Creek WMA	12	12	7	0.83 ± 0.10	65	0.023 ± 0.013	-	-	-	-	-	-	-	-
Joe Kurz WMA	30	12	5	0.85 ± 0.07	63	0.033 ± 0.018	30	8.11	6.07	10.22 ± 4.09	0.62 ± 0.17	0.72 ± 0.18	10	19
West Point WMA	5	5	3	0.70 ± 0.22	23	0.014 ± 0.009	-	-	-	-	-	-	-	-
Central GA Group	42	24	11	0.82 ± 0.07	36	0.009 ± 0.004	30	8.44	6.27	9.78 ± 4.93	0.61 ± 0.23	0.73 ± 0.18	10	20
BF Grant WMA	30	12	5	0.74 ± 0.12	24	0.009 ± 0.005	30	8.44	6.27	9.78 ± 4.94	0.61 ± 0.23	0.73 ± 0.19	10	20
Cedar Creek WMA	12	12	7	0.89 ± 0.08	19	0.009 ± 0.005	-	-	-	-	-	-	-	-
Chickasawhatchee WMA	30	10	4	0.78 ± 0.09	51	0.032 ± 0.017	30	6.78	5.24	7.89 ± 4.26	0.40 ± 0.19	0.612 ± 0.26	10	18
Clark Hill WMA	12	12	3	0.53 ± 0.14	54	0.032 ± 0.017	-	-	-	-	-	-	-	-
Cohutta WMA	10	10	4	0.71 ± 0.12	70	0.029 ± 0.016	10	5.89	5.89	7.78 ± 4.32	0.63 ± 0.32	0.66 ± 0.23	10	12
Dawson Forest WMA	28	11	3	0.65 ± 0.11	48	0.030 ± 0.016	28	7.33	5.76	8.89 ± 4.65	0.60 ± 0.14	0.67 ± 0.20	10	17
Dixon Meomorial WMA	30	10	4	0.78 ± 0.09	62	0.041 ± 0.022	30	8.00	5.90	9.44 ± 4.90	0.55 ± 0.22	0.66 ± 0.27	10	11
Fort Yargo SP	10	10	4	0.53 ± 0.18	56	0.034 ± 0.019	10	5.67	5.67	7.11 ± 4.10	0.68 ± 0.26	0.70 ± 0.12	-	-
Lake Russell WMA	30	12	5	0.81 ± 0.07	80	0.051 ± 0.027	30	6.89	5.03	8.33 ± 3.97	0.53 ± 0.19	0.65 ± 0.22	10	16
Ossabaw Island WMA	27	10	2	0.20 ± .015	3	0.001 ± 0.001	27	6.33	6.74	8.78 ± 4.35	0.55 ± 0.22	0.66 ± 0.22	10	9
Riverbend WMA	22	10	5	0.82 ± 0.10	131	0.091 ± 0.049	22	8.33	6.65	9.78 ± 4.52	0.71 ± 0.22	0.75 ± 0.19	10	15

Table 3.3. Pairwise F_{ST} and R_{ST} values. Pairwise F_{ST} values for mtDNA for each sampling site pair are above the diagonal, and pairwise R_{ST} values for all microsatellite-genotyped population pairs are above the diagonal. Significant values ($p<0.05$) are in bold.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Berry College WMA	1		NA	NA	**0.0350**	NA	**0.0320**	NA	NA	-0.0160	0.0050	**0.0430**	0.0310	**0.0550**	-0.0190	**0.0730**	0.0300
Big Lazer WMA	2	**0.4219**		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blanton Creek WMA	3	**0.4818**	0.0773		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Joe Kurz WMA	4	**0.3864**	0.0854	0.0055		NA	**0.0290**	NA	NA	0.0220	-0.0100	**0.0360**	0.0040	**0.0410**	-0.0440	**0.0620**	-0.0080
West Point WMA	5	**0.5324**	-0.0355	0.0341	0.0479		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BF Grant WMA	6	**0.6478**	**0.1392**	0.0430	**0.1365**	0.1452		NA	NA	0.0300	-0.0030	**0.0220**	0.0010	0.0110	-0.0150	-0.0030	0.0310
Cedar Creek WMA	7	**0.6517**	0.1352	**0.1195**	**0.1366**	0.0912	0.0602		NA	NA	NA	NA	NA	NA	NA	NA	NA
Clarks Hill WMA	8	**0.4096**	**0.5384**	**0.4931**	**0.3774**	**0.5433**	**0.6511**	**0.6521**		NA	NA	NA	NA	NA	NA	NA	NA
Cohutta WMA	9	**0.6380**	**0.5911**	**0.5393**	**0.4677**	**0.5721**	**0.6783**	**0.6827**	**0.5544**		-0.0330	-0.0020	-0.0390	-0.0020	**0.0990**	0.0060	**0.0830**
Dawson Forest WMA	10	**0.5386**	**0.6123**	**0.5419**	**0.4295**	**0.6062**	**0.7001**	**0.7037**	0.1135	**0.5438**		0.0000	-0.0120	0.0090	-0.0450	0.0170	-0.0020
Lake Russell WMA	11	**0.4803**	**0.4763**	**0.4196**	**0.3454**	**0.4362**	**0.5518**	**0.5610**	**0.2987**	**0.2645**	**0.2570**		-0.0140	-0.0070	-0.0060	0.0160	0.0320
Chickasawhatchee WMA	12	**0.4628**	**0.2073**	**0.1344**	0.0508	0.1839	**0.2735**	**0.2992**	**0.4698**	**0.3785**	**0.5040**	**0.3318**		-0.0010	-0.0310	0.0090	0.0030
Dixon Memorial WMA	13	**0.4062**	**0.1493**	0.0785	-0.0319	0.0784	**0.2171**	**0.1970**	**0.2926**	**0.4161**	**0.3218**	**0.2747**	**0.0997**		0.0070	-0.0140	**0.0390**
Fort Yargo State Park	14	**0.4728**	**0.5540**	**0.4695**	**0.3539**	**0.5383**	**0.6501**	**0.6550**	0.0701	**0.4993**	0.0259	**0.2295**	**0.4415**	**0.2620**		0.0180	-0.0170
Ossabaw Island WMA	15	**0.7810**	**0.8659**	**0.8082**	**0.7100**	**0.9341**	**0.9337**	**0.9369**	**0.3688**	**0.7793**	**0.2779**	**0.4805**	**0.7735**	**0.6404**	**0.1796**		**0.0620**
Riverbend WMA	16	**0.3973**	**0.2346**	**0.1643**	0.0405	0.1867	**0.3173**	**0.3038**	**0.2258**	**0.4074**	**0.2372**	**0.2529**	**0.1672**	-0.0657	**0.1920**	**0.5679**	

Table 3.4. Analysis of molecular variance (AMOVA) of mtDNA CR in GA WTD. A selection of analyses performed using *a priori* grouping scenarios based on geographic proximity. Significant values are indicated (***p<0.001, **p<0.01, *p<0.05).

	Grouping	Source of variation	d.f.	% variation	
A	[BF, CC]	Among groups	14	46.95	***
	All other sites separated	Among sites within groups	1	-1.94	ns
		Among individuals within sites	163	54.99	*
B	[JK, BC, WP, BL]	Among groups	12	43.84	***
	All other sites separated	Among sites within groups	3	1.91	ns
		Among individuals within sites	163	54.25	**
C	[BF, CC], [JK, BC, WP, BL]	Among groups	11	45.21	***
	All other sites separated	Among sites within groups	4	0.81	ns
		Among individuals within sites	163	53.98	***
D	[BF, CC], [JK, BC, WP, BL]	Among groups	10	43.43	***
	[CH, DM]	Among sites within groups	5	2.7	*
	All other sites separated	Among individuals within sites	163	53.87	***
E	[BF, CC], [JK, BC, WP, BL]	Among groups	9	42.99	***
	[CH, DM, RB]	Among sites within groups	6	3.45	ns
	All other sites separated	Among individuals within sites	163	53.56	***
F	[BF, CC], [JK, BC, WP, BL]	Among groups	10	45.95	***
	[DM, RB]	Among sites within groups	5	0.27	ns
	All other sites separated	Among individuals within sites	163	53.79	***
G	[BF, CC, JK, BC, WP, BL]	Among groups	8	44.4	***
	[CH, DM, RB]	Among sites within groups	7	3.71	*
	All other sites separated	Among individuals within sites	163	51.9	***
H	[BF, CC, JK, BC, WP, BL]	Among groups	7	46.68	***
	[DM, RB]	Among sites within groups	8	1.25	ns
	All other sites separated	Among individuals within sites	163	52.07	***
I	[BF, CC, JK, BC, WP, BL]	Among groups	8	46.62	***
	[DM, RB], [DF, FY]	Among sites within groups	7	1.5	ns
	All other sites separated	Among individuals within sites	163	51.88	***
J	[BF, CC, JK, BC, WP, BL]	Among groups	7	45.98	***
	[DM, RB], [DF, FY, CL]	Among sites within groups	8	2.52	ns
	All other sites separated	Among individuals within sites	163	51.5	***
K	[BF, CC, JK, BC, WP, BL, FY]	Among groups	7	33.49	***
	[DM, RB], [DF, CL]	Among sites within groups	8	14.51	***
	All other sites separated	Among individuals within sites	163	52	***
L	[BF, CC, JK, BC, WP, BL]	Among groups	6	34.42	***
	[DM, RB], [CO, DF, FY, CL]	Among sites within groups	9	13.55	***
	All other sites separated	Among individuals within sites	163	52.02	***
M	[BF, CC, JK, BC, WP, BL]	Among groups	6	45.09	***
	[DM, RB], [DF, FY, CL, OI]	Among sites within groups	9	3.88	*
	All other sites separated	Among individuals within sites	163	51.02	***

Table 3.5. Proportion of membership of white-tailed deer from 11 Georgia sampling sites in inferred clusters. Clustering in STRUCTURE under the admixture model with user define k values (K=5, 10). No *a priori* population designations were used in calculations. Shaded values highlight proportions greater than 3x (dark grey; >0.60 (K=5), > 0.3 (K=10)) and 2x (light grey; >0.4 (K=5), > 0.2 (k=10)) the assigned membership proportion expected from random sampling for each k value (0.20 (k=5), 0.1 (k=10).

A

		Inferred clusters (K=5)				
	N	1	2	3	4	5
Berry College WMA	28	0.060	0.606	0.139	0.081	0.112
BF Grant WMA	30	0.191	0.279	0.203	0.086	0.241
Chickasawhatchee WMA	30	0.638	0.070	0.109	0.082	0.101
Cohutta WMA	10	0.092	0.055	0.671	0.059	0.123
Dawson Forest WMA	28	0.110	0.140	0.348	0.191	0.211
Dixon Memorial WMA	30	0.162	0.117	0.105	0.123	0.493
Fort Yargo SP	10	0.218	0.313	0.201	0.058	0.210
Joe Kurz WMA	30	0.181	0.299	0.197	0.114	0.209
Lake Russell WMA	30	0.176	0.085	0.388	0.156	0.195
Ossabaw Island WMA	27	0.060	0.037	0.070	0.791	0.042
Riverbend WMA	22	0.248	0.091	0.180	0.080	0.401

B

		Inferred clusters (k=10)									
	N	1	2	3	4	5	6	7	8	9	10
Berry College WMA	28	0.054	0.131	0.037	0.047	0.047	0.066	0.042	0.508	0.043	0.026
BF Grant WMA	30	0.060	0.082	0.065	0.260	0.128	0.057	0.035	0.115	0.134	0.064
Chickasawhatchee WMA	30	0.055	0.027	0.074	0.031	0.076	0.105	0.043	0.016	0.056	0.518
Cohutta WMA	10	0.041	0.140	0.584	0.034	0.035	0.019	0.017	0.026	0.049	0.055
Dawson Forest WMA	28	0.083	0.151	0.225	0.097	0.112	0.096	0.074	0.036	0.094	0.033
Dixon Memorial WMA	30	0.411	0.033	0.029	0.056	0.057	0.214	0.058	0.034	0.076	0.032
Fort Yargo SP	10	0.047	0.031	0.058	0.107	0.255	0.163	0.023	0.105	0.150	0.062
Joe Kurz WMA	30	0.052	0.063	0.109	0.167	0.127	0.138	0.041	0.112	0.146	0.045
Lake Russell WMA	30	0.065	0.318	0.130	0.110	0.072	0.095	0.061	0.031	0.072	0.047
Ossabaw Island WMA	27	0.026	0.057	0.037	0.019	0.027	0.025	0.697	0.022	0.033	0.058
Riverbend WMA	22	0.060	0.043	0.106	0.083	0.272	0.048	0.023	0.026	0.285	0.053

Table 3.6. MHC class I heavy chain alleles in GA WTD by sampling site. Allele frequencies expressed as proportions of alleles identified at each sampling site in GA, with 27 distinct alleles total.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
BF Grant WMA	0.04	0.01	0.02	0.13	0.02	0.01	0.23	0.01	0.01	0.04	0.01	0.01	0.02	0.01		0.03	0.24		0.01	0.12		0.03		0.01			
Chickasawhatchee WMA	0.01		0.03	0.03			0.13			0.05	0.06	0.01	0.01	0.04	0.01	0.12	0.21	0.03	0.01	0.12		0.05		0.07			0.01
Dawson Forest WMA				0.05			0.01			0.03	0.02	0.02	0.02	0.08	0.03	0.06	0.36	0.01	0.02	0.14	0.02	0.03		0.05	0.03		
Joe Kurz WMA	0.01	0.01		0.13	0.01	0.02	0.11			0.03	0.03	0.02			0.03	0.07	0.22	0.01	0.04	0.1	0.02	0.05		0.08	0.02		
Lake Russell WMA			0.01	0.21	0.02		0.01			0.03	0.01				0.01	0.11	0.11		0.06	0.35	0.02	0.03		0.01		0.02	0.01
Cohutta WMA	0.04	0.04			0.04		0.04				0.07		0.07		0.04	0.04	0.15			0.26				0.15	0.07		
Dixon Memorial WMA				0.04		0.02	0.08				0.1			0.06		0.02	0.18			0.12			0.33	0.04	0.02		
Ossabaw Island WMA				0.22			0.06			0.03	0.03						0.22	0.08	0.11	0.22		0.03					
Riverbend WMA		0.06	0.06	0.06	0.06	0.06			0.06	0.12		0.06		0.06	0.06		0.06			0.12	0.06					0.06	0.06

Figure 3.1. GA HD mortality and morbidity indices and project sampling sites. Blue polygons represent the boundaries of areas where deer were sampled during the 2005 and 2006 hunting seasons.

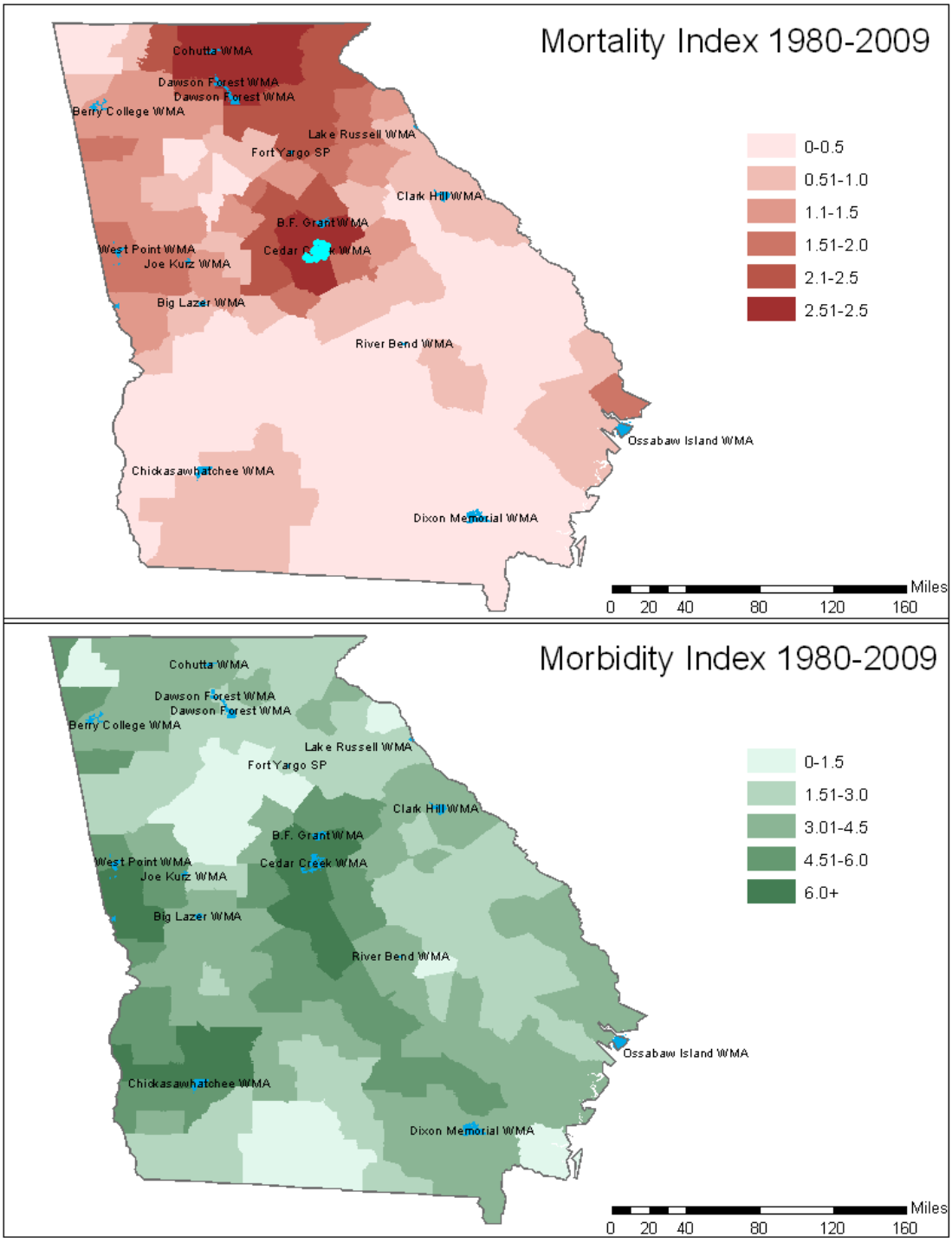


Figure 3.2. GA WTD restocking locations. County dot-density representations of extrapolated descendants of introduced deer from Wisconsin (red, A,B), Eastern US (NC, KY, MD, purple, C,D), and Texas (green, E,F). Mortality (A, C, E) and morbidity (B, D, F) are presented for comparison (see Fig 3.1 for index values). (1 dot= 1 animal, see text for extrapolation method).

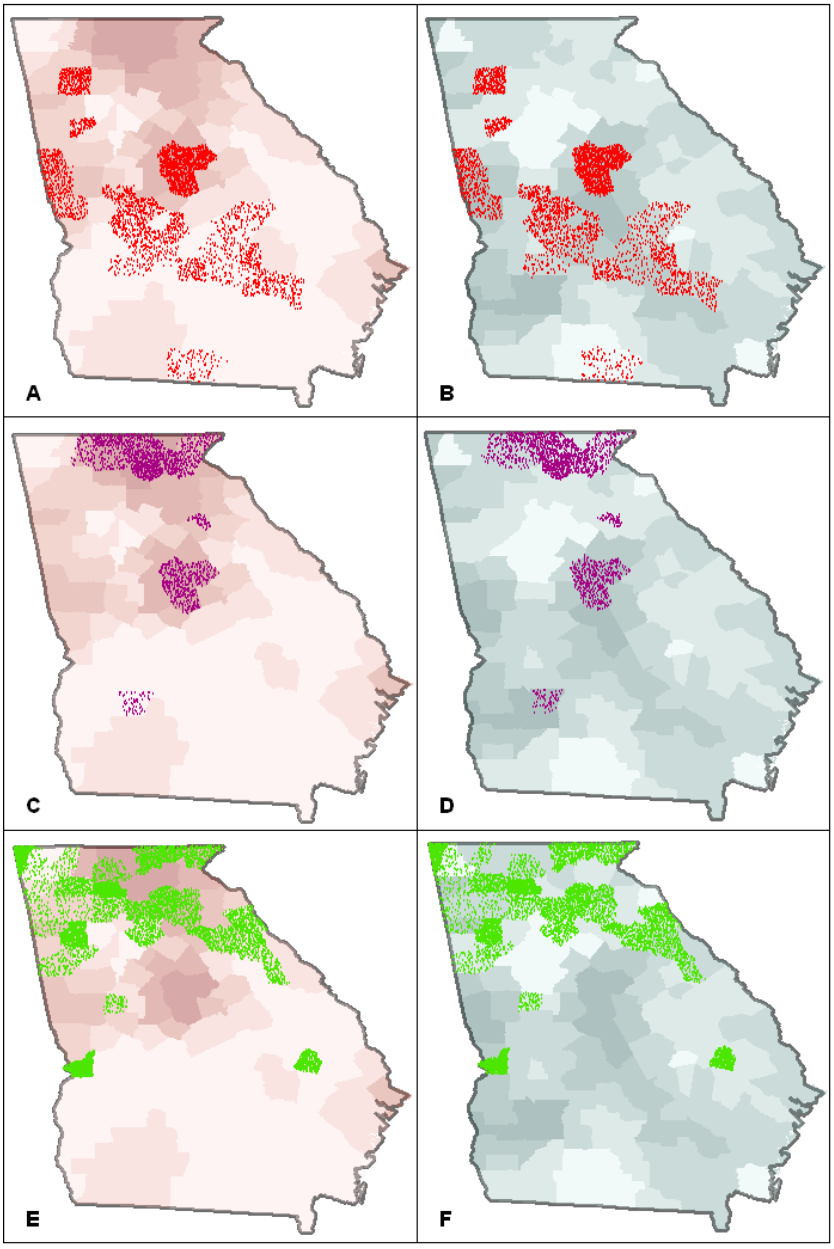
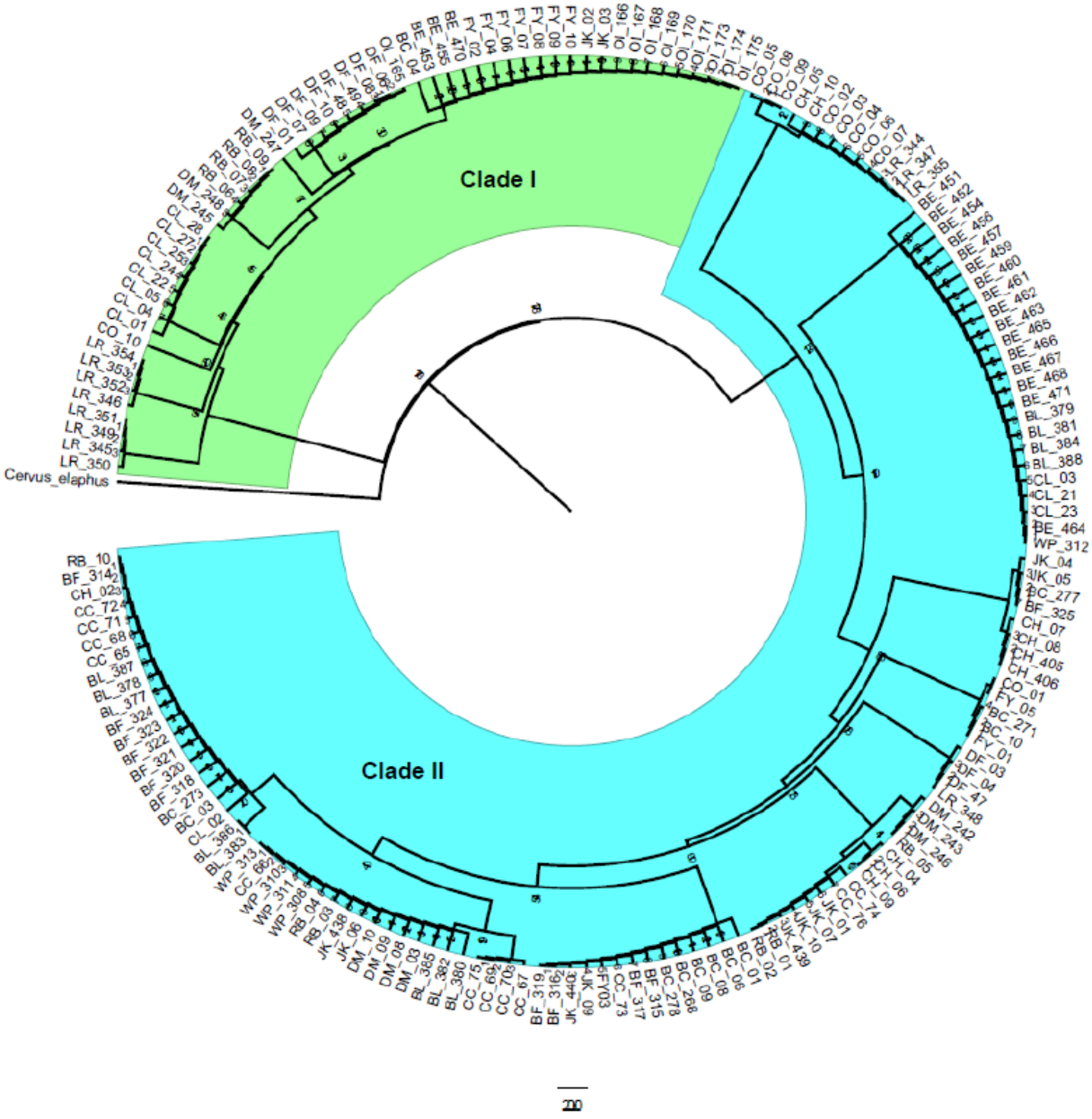


Figure 3.3. Neighbor-joining phylogenetic tree of GA WTD. Clades I and II are highlighted, and the tree is rooted using elk (*Cervus elaphus*) mtDNA sequence (A). A 3-dimensional visualization of an unrooted N-J tree mapped over sampling sites in GA (B).

A



B

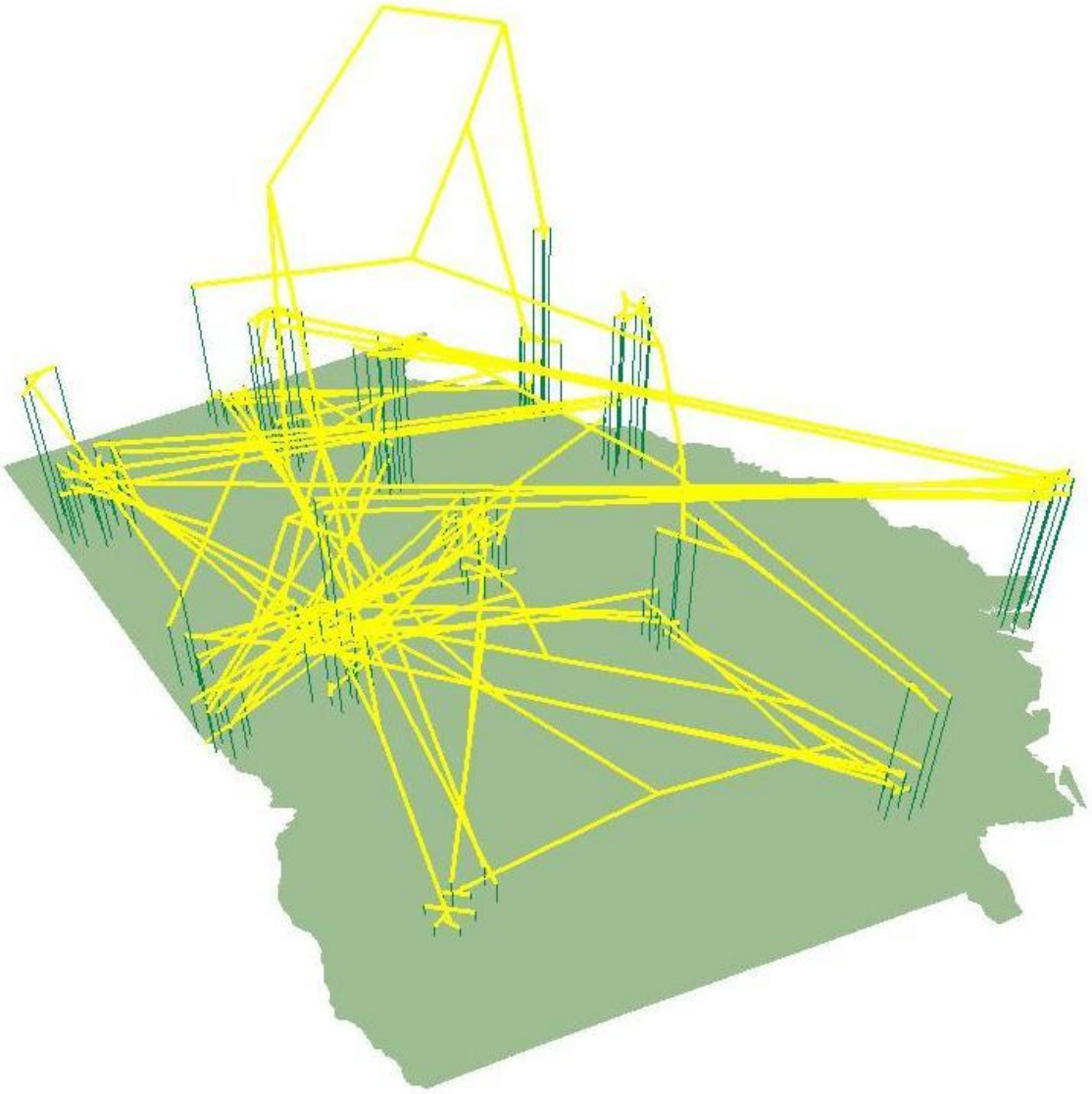


Figure 3.4. Proposed population structure maximizing among-group variation by AMOVA calculations of mtDNA CR. Circled sites represent group selections maximizing among-group variation while maintaining non-significant within-group variance (calculations presented in Table 3.3).

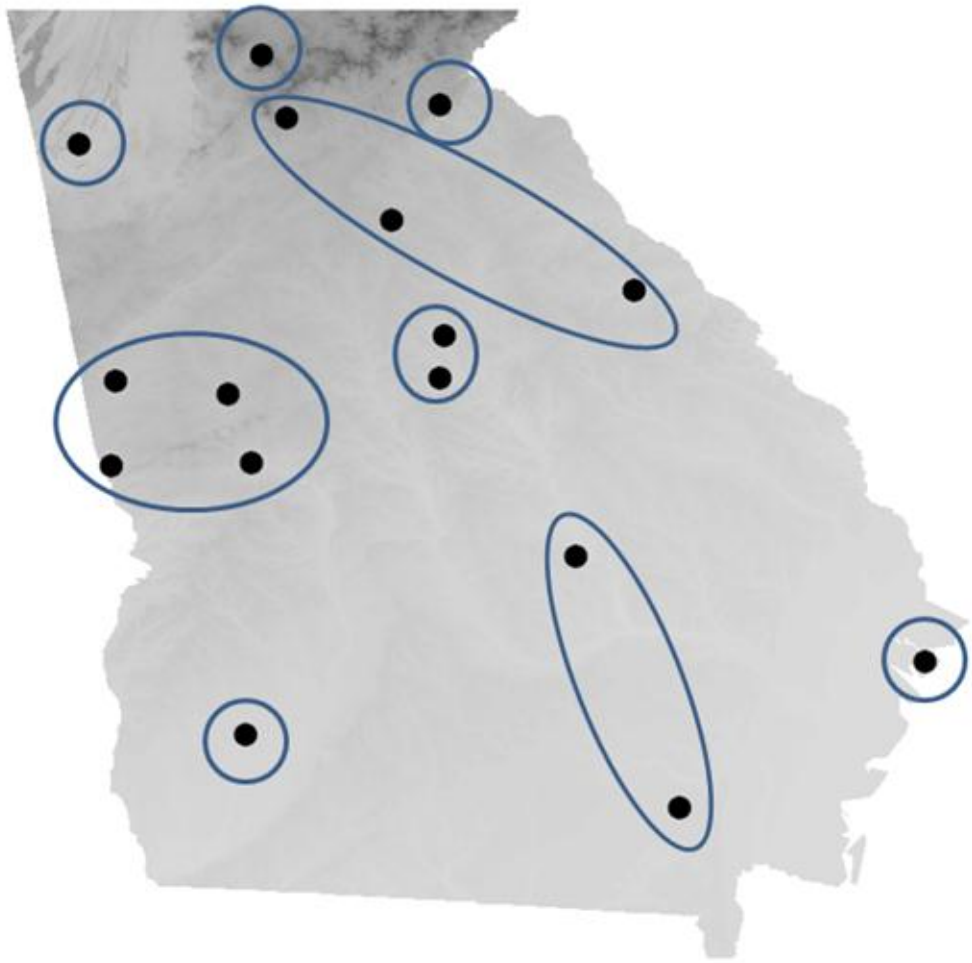


Figure 3.5. WTD mtDNA CR haplogroups in Georgia. Haplotype diversity of WTD sampled across Georgia. Pie chart sizes are proportional to sample size, with wedges representing haplotypes and colors corresponding to haplogroups (A). A median-joining network of mtDNA CR haplotypes calculated and constructed using NETWORK v4.6, with 10 haplogroups identified (B). This network incorporates 21 WI WTD sequences identified in Figure 3.5, including the single outlier shown here not included in a haplogroup.

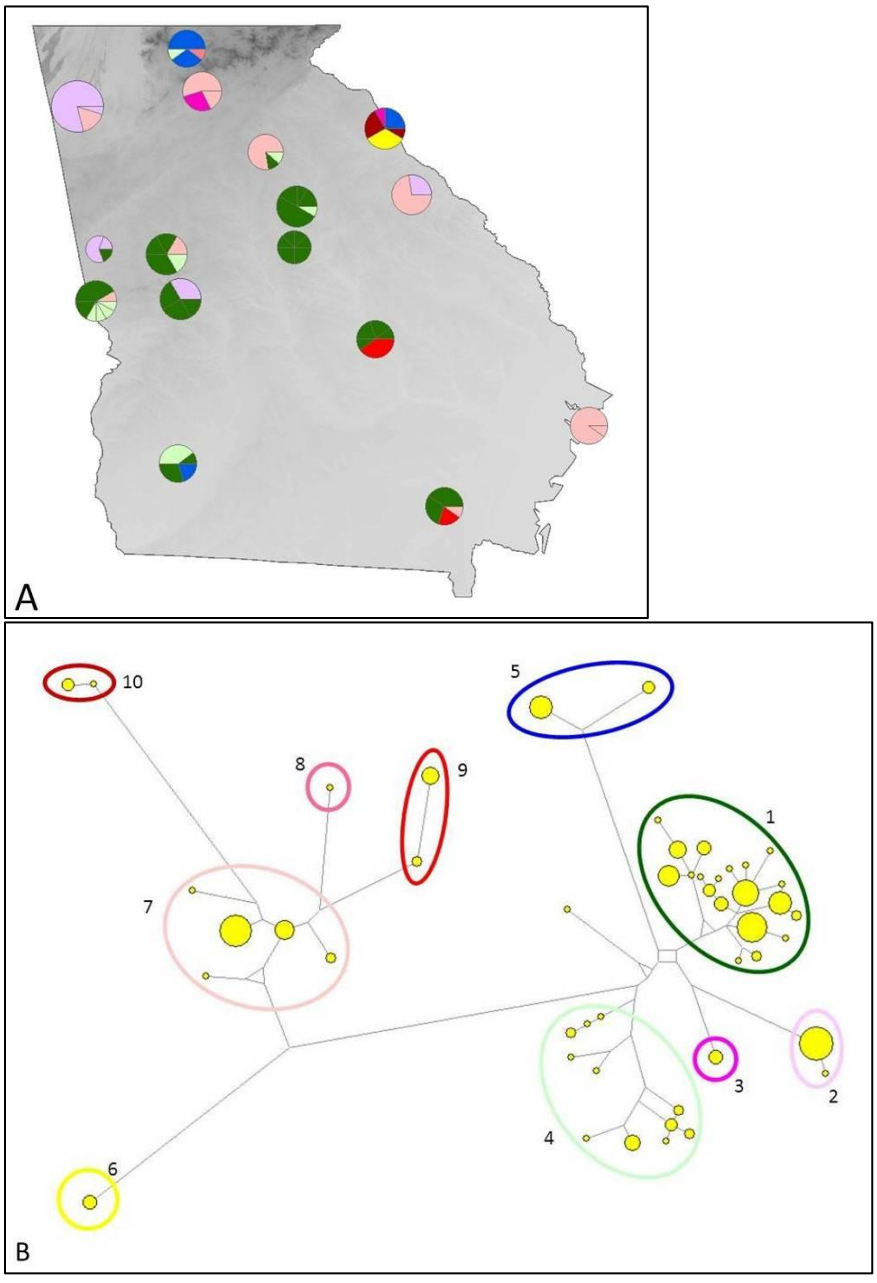


Figure 3.6. mtDNA CR haplotypes compared between GA and WI WTD. Locations of WI WTD released in GA, with dot densities of modern descendants normalized to account for disparities in release dates between 1944-1962 (see text for method). Each dot represents 1 extrapolated contemporary descendant. Pie chart size is proportional to sample size of GA WTD, black wedges correspond to the portion of samples sharing identical haplotypes with deer sampled from WI (A). A M-J network of GA and WI WTD haplotypes. Shaded nodes represent WI haplotypes found only in WI WTD samples, black nodes represent haplotypes found in both GA and WI WTD (B).

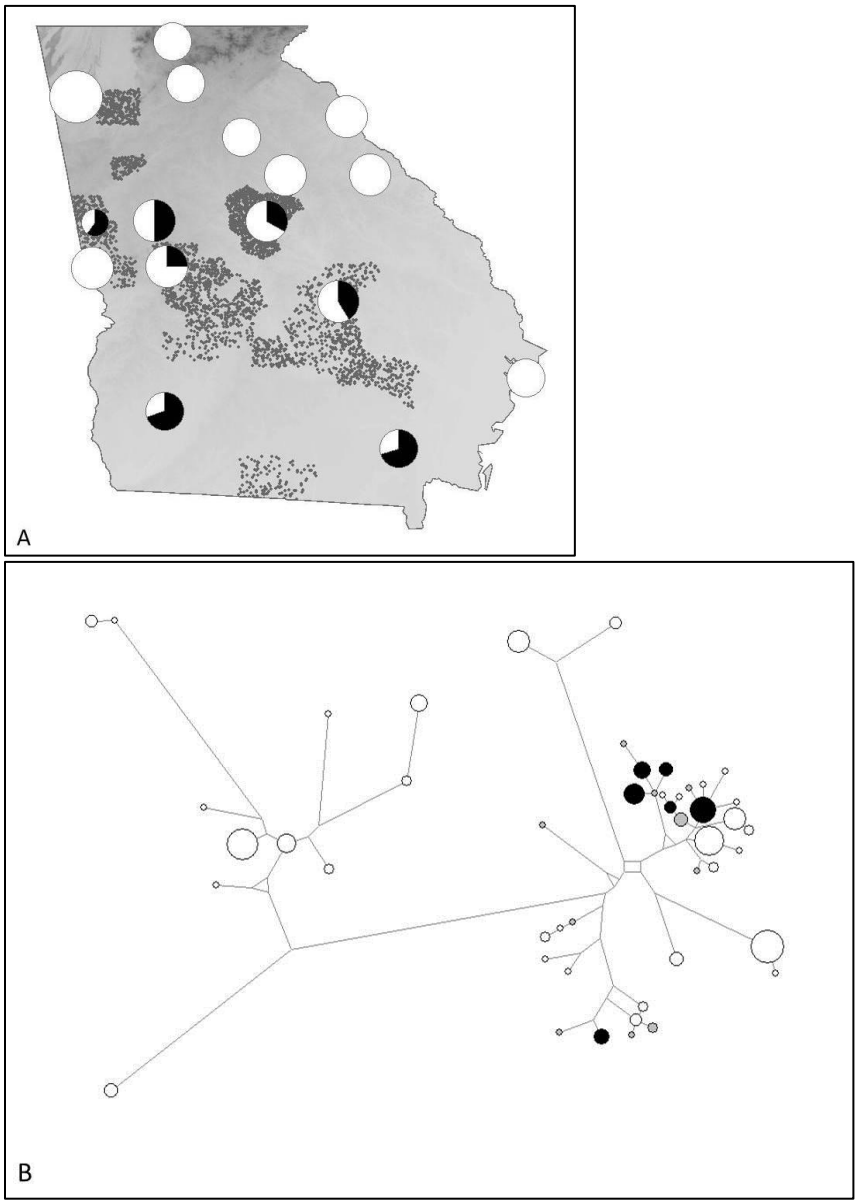


Figure 3.7. Microsatellite allele frequency by locus. A graphic view of allele frequency and distribution, highlighting disparate allelic diversity between loci, constructed in GenAlEx v.6. Berry College WMA (BE), BF Grant WMA (BF), Chickasawhatchee WMA (CH), Cohutta WMA (CO), Dawson Forest WMA (DF), Dixon Memorial WMA (DM), Fort Yargo SP (FY), Joe Kurz WMA (JK), Lake Russell WMA (LR), Ossabaw Island WMA (OI), Riverbend WMA (RB).

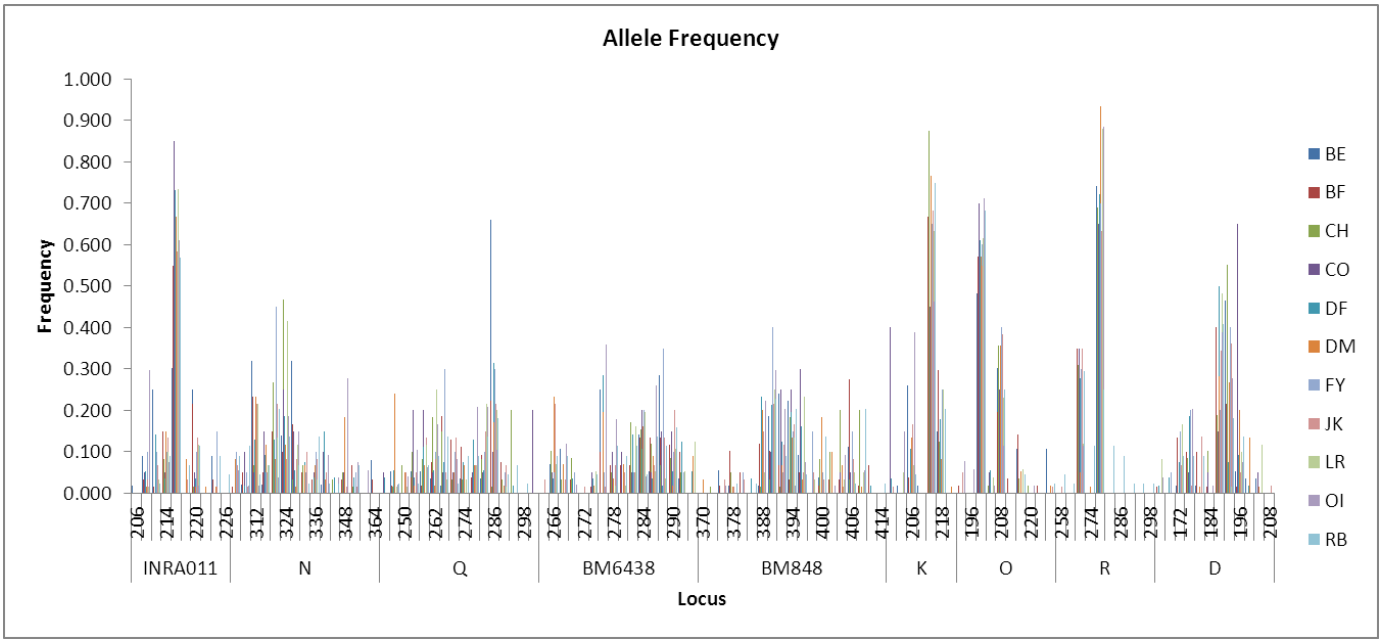


Figure 3.8. Mean *Ln Pr(X|K)* values over 5 iterations at K=1-12.

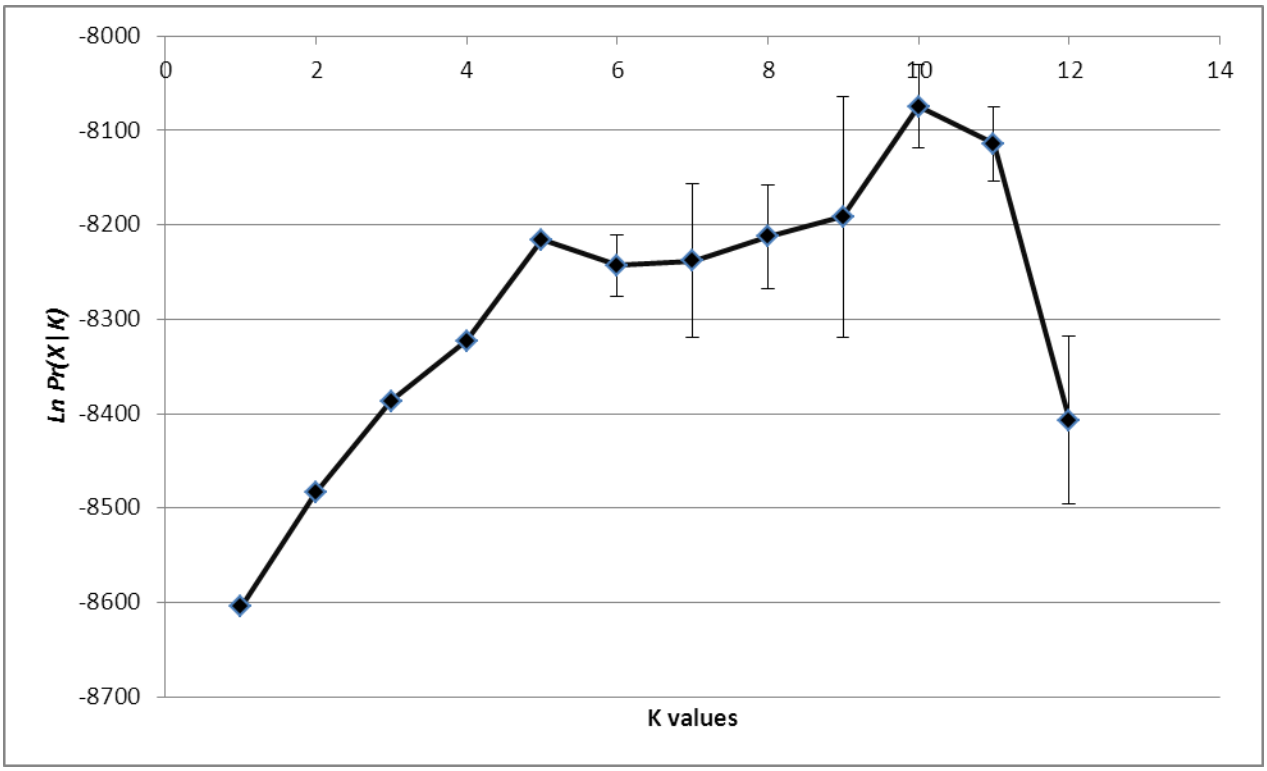
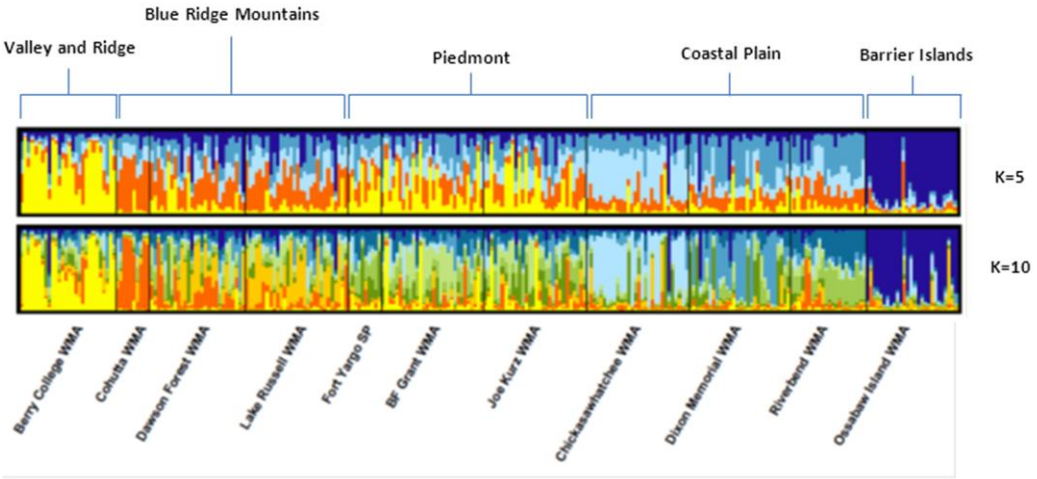


Figure 3.9. Assignment of GA WTD to K inferred populations (K=5,10). Assignments to clusters were performed with no *a priori* population definitions beyond designating K. Each individual is represented by a vertical bar proportionately partitioned into K=5 and 10 respectively. Colors correspond to assigned clusters, and black lines separate sampling sites. The Georgia physiogeographic region where each site is located is identified (A). A map depicting the 5 physiogeographic regions of Georgia (blue=Coastal plain, green=Piedmont, red=Blue Ridge Mountains, yellow=Valley and Ridge, cream=Appalachian Plateau. Barrier Islands are along the coastline to the southeast). Sampling sites are labeled (B).



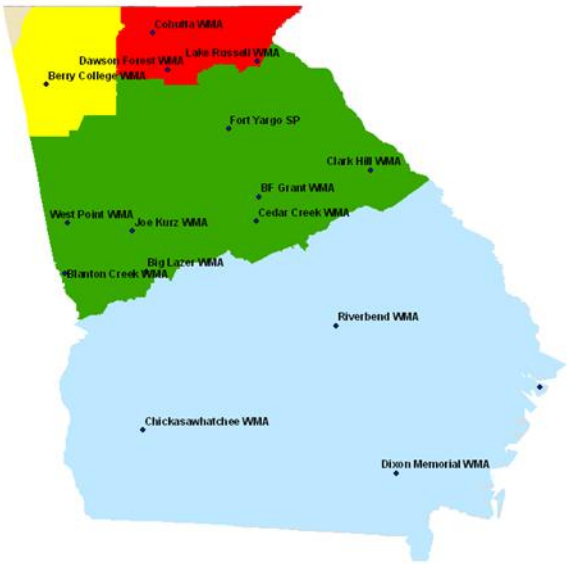
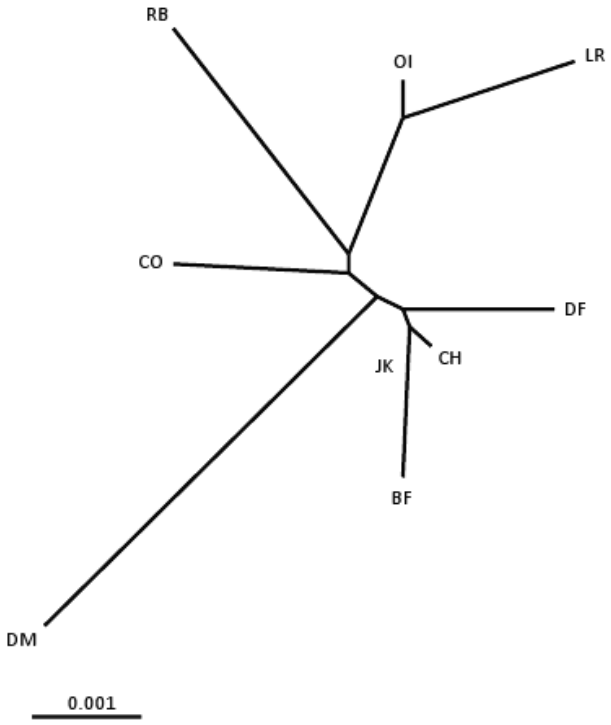


Figure 3.10. Un-rooted phylogenetic tree analysis of sampling sites based on MHC alleles.



CHAPTER 4

SPATIAL HETEROGENEITY IN WHITE-TAILED DEER POPULATION GENETICS: A COMPARISON OF RESTOCKED AND NATIVE POPULATIONS[1]

[1] McGraw, S.N., Robinson, S., Jones, K., Stallknecht D., and Howerth, E. 2011. To be submitted to *Journal of Wildlife Management*

ABSTRACT

Following the near extirpation of white-tailed deer (*Odocoileus virginianus*, WTD) from the southeastern United States by the early 1900s, an extensive recovery program was implemented that included the introduction of thousands of deer from widely distant areas. Population genetic studies in extant southeastern deer have found high genetic variability, which has been attributed to restocking efforts. However, evaluations of the success of the recovery program have lead some authors to conclude that introduced deer may have contributed minimally to modern herds, and that genetic variability is due instead to evolutionary processes common to other southeastern taxa. Wisconsin represents an area where WTD numbers were diminished but recovered without a restocking program. We compared the population structure and overall genetic variability of deer across comparably sized areas of northern Georgia and southern Wisconsin to determine the differences observable between a restocked and non-restocked location. We found significantly higher genetic variability in Georgia deer based on pairwise nucleotide diversities identified in sequence data derived from the mitochondrial D-loop control region ($p<0.01$). Population structure estimated by pairwise Wright's F_{ST} calculations found more than 80% of pairwise comparisons between Georgia sampling sites (N=12) to be significantly different, while less than 4% of comparisons between WI sampling sites (N=11) were significantly different ($p<0.05$). We estimate fine-scale population genetic structuring in Georgia WTD that is not apparent in Wisconsin WTD, which is consistent with the influence of introduced genetic material during restocking efforts.

INTRODUCTION

Arguably the most successful species recovery effort in a large mammal was implemented in response to the near extirpation of white-tailed deer (*Odocoileus virginianus*, WTD) in the southeastern United States (US) by the early 20th century. The conservation program incorporated three general strategies; strict protection laws, habitat management, and a restocking program (Smith, Baccus et al. 1984; Leberg and Ellsworth 1999). In Georgia alone, over 3400 deer were translocated between 1928-1975, of which more than 1500 deer were from out of state (Federal Aid in Wildlife Restoration Georgia State Game and Fish Commission 1975). Retrospective examination of these efforts across the southeast has led to differing conclusions regarding the relative value of reintroductions. Some authors have hypothesized that the rebound of this species was due primarily to habitat management and protection laws, and that restocked deer have contributed minimally to modern herds (Ellsworth, Honeycutt et al. 1994; Ellsworth, Honeycutt et al. 1994). These conclusions were based partially on similarities between the geographic intraspecific variation of mitochondrial DNA (mtDNA) of extant WTD and other native species. Such similarities suggest that the variation observed in WTD is due to historical events common to other taxa in the southeast, independent of introductions (Leberg, Stangel et al. 1994; Leberg and Ellsworth 1999). By comparing current populations likely to have been introduced from the same source, researchers have found evidence both in support of and refuting common ancestry (Leberg, Stangel et al. 1994; DeYoung, Demarais et al. 2003). These data suggest that some, if not all, translocations failed to significantly affect modern herds. However, numerous studies have demonstrated high spatial genetic variability in southeastern deer (Chesser, Smith et al. 1982; Smith, Baccus et al. 1984; Scribner, Smith et al. 1997; Purdue, Smith et al. 2000; Smith, Novak et al. 2001). These findings have been attributed to the

introduction of genetic material from widely distant areas (Karlin, Heidt et al. 1989; DeYoung, Demarais et al. 2003). However, to our knowledge, no published study has directly compared the genetic structure of a heavily reintroduced population to that of a population that recovered from historical bottlenecks without restocking. Wisconsin WTD numbers were severely reduced by the late 1800s in concordance with depletions across most of the US. However, though hunting was regulated, restocking efforts were not implemented as they were in the southeast. In this study, we will compare mtDNA sequence data from similarly sized areas of both Georgia and Wisconsin to evaluate the relative effects of restocking on recovered population genetics. We hypothesize that GA WTD will express significantly greater spatial genetic variability than WI WTD.

MATERIALS AND METHODS

Study Area

We compiled mitochondrial sequence data from white-tailed deer within comparably sized areas (approximately 20,000 sqmi, Figure 4.1) of northern Georgia (N=139, 12 sites) and southern Wisconsin (N=307, 11 sites).

Methods

All sequence data was originally amplified using primers specific to the white-tailed deer mitochondrial D-Loop control region (mtDNA CR) (McGraw 2011, Robinson 2011, GENBANK). We aligned sequences from all individuals (N=478) and an out-group (elk, *Cervus elaphus*, GENBANK Accession #GU457434) in CLC SEQUENCE VIEWER (alignment parameters: gap open cost =20, gap extension cost=0, end gap cost=free, www.clcbio.com). As individual sequences from each state were originally amplified with different primers pairs, we trimmed

compiled sequences to the overlapping segment (500bp) prior to analysis. Using the neighbor-joining method in MEGA v.4 (Tamura, Dudley et al. 2007), we conducted bootstrap tests of phylogeny for each state under the maximum composite likelihood model (1000 replications), and visualized them using FIGTREE v.1.1 (tree.bio.ed.ac.uk/software/figtree). We used the same outgroup (*C. elaphus)* to root both trees, and maintained all other parameters between each analysis constant (program default options unless otherwise noted). Using ARLEQUIN v.3.5.1.2 (Excoffier, Laval et al. 2005), we identified distinct haplotypes in each state, their frequencies, and calculated molecular diversity statistics in each state. Also in ARLEQUIN, we compared the genetic distance between pairs of sampling sites in each state using both pairwise F_{ST} and corrected pairwise nucleotide differences (accounting for within-site variability). For pairwise comparisons between sampling sites and network analyses, sequences were screened for ambiguous base calls which would preclude definitive haplotype assignment. The sequences included in population comparisons beyond bootstap tests of phylogeny were 139 in GA and 180 from WI. We constructed median-joining networks for haplotypes in each state in NETWORK v.4.6 (www.fluxus-engineering.com). Geographic (km) distances between sampling sites were measured in ArcGIS and compared to genetic distances based on pairwise nucleotide differences.

RESULTS

Of the 500bp segment of mtDNA CR analyzed, 113 (GA) and 55 (WI) loci were polymorphic. The total number of haplotypes identified in the sampling region of each state was 32 (GA) and 28 (WI). The number of haplotypes identified per site in GA ranged from 3 to 7 (mean=4.4), while the range in WI was 7 to 12 (mean=9,Table 4.1). The average haplotypic diversity by sampling site was significantly less (p=0.002) in Georgia (0.70) than in Wisconsin (0.80) by student's t-test (unpaired, assuming unequal variance). The average nucleotide diversity across

all samples from each state was 0.0541 (±0.026, GA) and 0.0195 (±0.010, WI). Average nucleotide diversity by site was significantly greater (p=0.004) in Georgia (0.30) than in Wisconsin (0.02) by student's t-test (unpaired, assuming unequal variance). Of 66 pairwise comparisons between sampling sites in GA, 53 (80.3%) were significantly different and in WI, of 45 pairwise comparisons, 3 (6.67%) were significantly different ($p<0.05$, population pairwise F_{ST}, 3024 permutations; Table 4.2). Neighbor-joining phylogenetic trees are presented in Figure 4.2. Network analyses of haplotypes in each state are presented in Figure 4.3. Comparison of geographic and genetic distances in each state found a poor correlation in both GA (r^2=-0.165) and WI (r^2=-0.00008, Figure 4.4).

DISCUSSION

The composite population structure across each studied region demonstrated significantly greater global genetic variability in northern Georgia than southern Wisconsin, based on bootstrap tests of phylogeny (Figure 4.2) and haplotypic and nucleotide diversities ($p<0.01$). The total number of haplotypes identified in each state is similar (GA=32, WI=28), but haplotype sequences were more genetically distant in GA than in WI based on the total number of polymorphic sites (GA=113, WI=55). Also, haplotypes in WI are shared more equally across the study area than in GA, which is exemplified by the higher per site haplotypic diversity observed in WI (Table 4.1). This pattern of extensively shared haplotypes contributes to the limited differences found between sampling sites in northern WI. Overall, pairwise differences between sampling sites in each state support significant fine-scale geographic structure in GA deer that is not apparent in WI deer. Comparison of geographic and genetic distances in GA deer demonstrates poor correlation, which would be expected if populations differ due to processes other than genetic drift alone (Wright 1943; Epperson 2003). These findings support our

hypothesis that GA WTD would demonstrate a higher degree of spatial genetic variability than WI deer, likely attributable to restocking history. Assuming deer introduced from geographically distant sites survived to significantly influence modern deer, a high degree of spatial heterogenetity would be expected, especially in mtDNA genes. Due to the matrilinear descent of mtDNA, population structure estimated using mtDNA markers is strongly biased by the limited dispersal of does (Perez-Espona, Perez-Barberia et al. 2010). Even fine-scale geographic differences in founder populations may remain apparent many generations later due to limited exchange of mtDNA genetics between groups (Leberg and Ellsworth 1999). The relatively low variability of WI WTD would be expected due to genetically similar native foundation stock shared between sampling sites, and a lack of recent introduction of diverse genetic information. Our findings confirm the anticipated differences in genetic structure observable between populations historically restocked and those recovered from historic bottlenecks without introductions. While we cannot unequivocally rule out other potential factors influencing the disparities in genetic variability in these states, the most readily apparent cause is differences in restocking history. Other potential factors may relate to other differences in biogeographic history between northern and southern WTD. Evidence of significantly greater genetic variability in a restocked population than a non-restocked population as seen in these sites supports the claim that deer introduced to the southeast contributed significantly to modern deer herds. However, these findings do not rule out the possibility that recovery programs would have been equally successful without a restocking component.

Table 4.1. Molecular diversity indices in Georgia and Wisconsin sampling sites. Total number of samples analyzed per population or group (N), number of haplotypes per site (n_h), haplotype (h) and nucleotide (π) diversities (± standard deviation, SD).

	Georgia WTD mtDNA CR						Wisconsin WTD mtDNA CR				
Site	*N*	n_h	*h* ± SD	n_{ps}	π ± SD	Site	*N*	n_h	*h* ± SD	n_{ps}	π ± SD
BC	12	7	0.833 ± 0.100	60	0.0301 ± 0.0162	A	22	9	0.883 ± 0.417	34	0.0152 ± 0.0082
BE	19	3	0.368 ± 0.125	36	0.0198 ± 0.0106	B	20	9	0.826 ± .0073	31	0.0188 ± 0.0100
BF	12	5	0.727 ± 0.113	26	0.0121 ± 0.0070	C	9	7	0.917 ± 0.092	33	0.0243 ± 0.0138
BL	12	4	0.803 ± 0.063	23	0.0207 ± 0.0114	D	28	11	0.900 ± 0.029	41	0.0205 ± 0.0107
CC	12	7	0.879 ± 0.075	16	0.0095 ± 0.0056	E	13	8	0.910 ± 0.056	36	0.0203 ± 0.0111
CL	12	3	0.530 ± 0.136	48	0.0377 ± 0.0201	F	20	9	0.790 ± 0.086	36	0.0140 ± 0.0077
CO	10	4	0.711 ± 0.118	66	0.0368 ± 0.0200	G	17	10	0.919 ± 0.043	38	0.0228 ± 0.0122
DF	11	3	0.655 ± 0.112	42	0.0357 ± 0.0192	H	20	12	0.879 ± 0.065	34	0.0195 ± 0.0104
FY	10	4	0.533 ± 0.180	54	0.0419 ± 0.0227	I	9	7	0.917 ± 0.092	29	0.0196 ± 0.0113
JK	12	5	0.849 ± 0.067	57	0.0406 ± 0.0216	J	19	8	0.866 ± 0.046	34	0.0248 ± 0.0131
LR	12	5	0.818 ± 0.070	74	0.0635 ± 0.0333						
WP	5	3	0.700 ± 0.218	21	0.0168 ± 0.0107						

Table 4.2. F_{ST} values by sampling site. Sampling site pairwise F_{ST} values for GA (A) and WI (B) below the diagonal. Above the diagonal, plus signs represent significantly different sampling site pairs ($p<0.05$).

A

	BC	BF	BL	CC	JK	WP	BE	CO	LR	CL	DF	FY
BC		-	-	+	-	-	+	+	+	+	+	+
BF	0.04236		+	-	+	-	+	+	+	+	+	+
BL	0.07680	0.12711		-	-	-	+	+	+	+	+	+
CC	0.12567	0.04193	0.14188		+	+	+	+	+	+	+	+
JK	0.00135	0.12567	0.07619	0.14034		-	+	+	+	+	+	+
WP	0.04128	0.14115	-0.02738	0.10859	0.04489		+	+	+	+	+	+
BE	0.46692	0.63476	0.41881	0.65830	0.37109	0.53292		+	+	+	+	+
CO	0.53903	0.67218	0.59699	0.69135	0.47009	0.57731	0.64302		+	+	+	+
LR	0.41965	0.54857	0.47959	0.56705	0.34427	0.43948	0.47893	0.26674		+	+	+
CL	0.48250	0.64008	0.53539	0.65799	0.37490	0.54253	0.41161	0.55524	0.29047		-	-
DF	0.53696	0.69476	0.61390	0.71300	0.43247	0.61103	0.54015	0.54620	0.24832	0.12675		-
FY	0.45971	0.64025	0.55235	0.66149	0.35382	0.53852	0.47168	0.50086	0.22255	0.08444	0.03822	

B

	A	B	C	D	E	F	G	H	I	J
A		-	-	-	-	-	-	-	-	+
B	-0.02717		-	-	-	-	-	-	-	-
C	0.05151	0.01612		-	-	+	-	-	-	-
D	-0.00847	-0.02093	-0.02893		-	-	-	-	-	-
E	-0.04027	-0.04620	-0.04058	-0.04908		-	-	-	-	-
F	-0.02479	-0.01777	0.11181	0.01576	-0.01526		-	-	-	+
G	0.01721	0.01158	-0.04183	-0.02240	-0.03382	0.05288		-	-	-
H	-0.01064	-0.01232	0.03353	-0.01439	-0.03443	-0.01328	-0.02002		-	-
I	-0.05133	-0.05947	-0.03343	-0.06121	-0.08256	-0.03325	-0.04445	-0.05324		-
J	0.10890	0.05959	-0.03832	0.01677	0.02460	0.13849	-0.00971	0.04599	0.01222	

Figure 4.1. Sampling site locations in GA and WI.

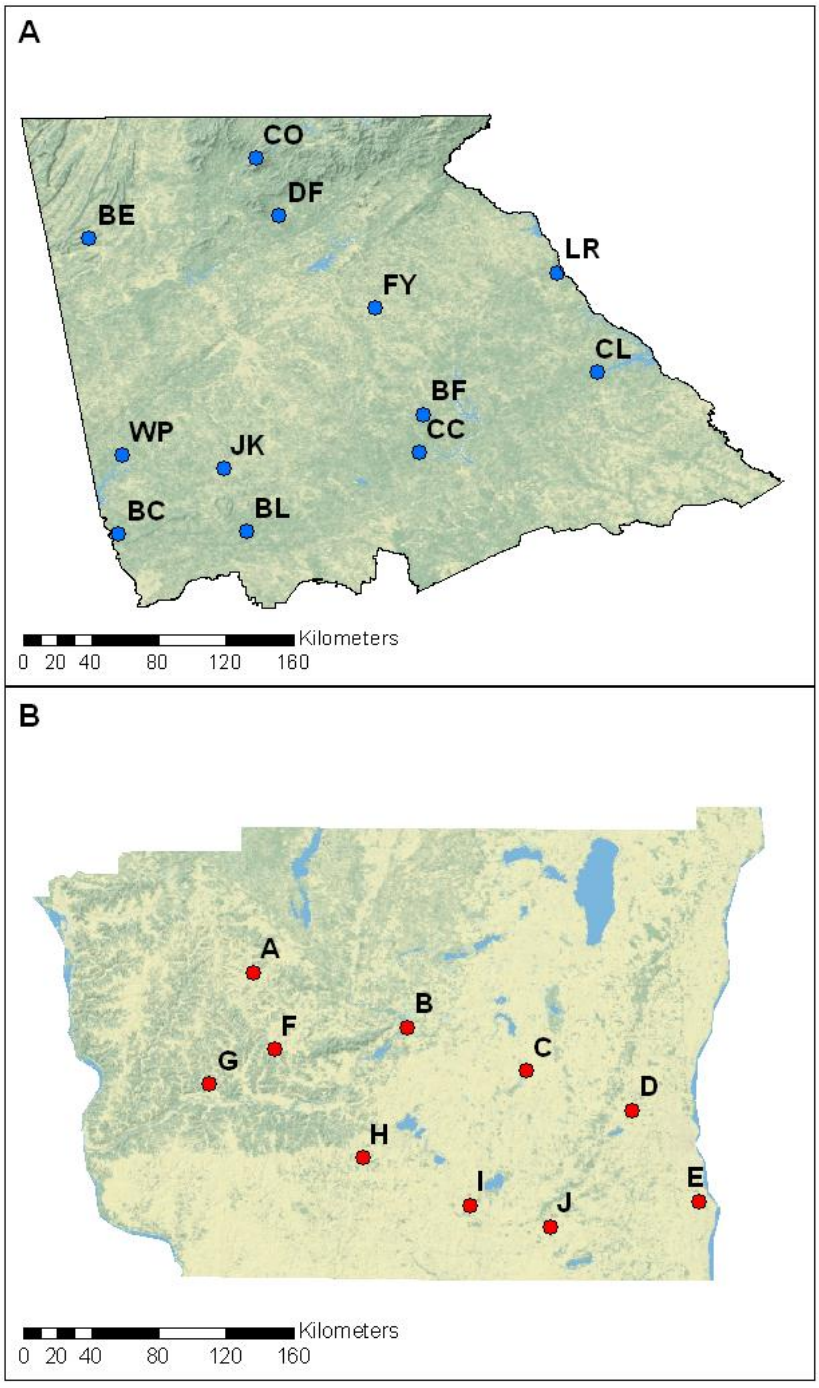


Figure 4.2. Phylogenetic analyses of GA and WI white-tailed deer (mtDNA CR). Bootstrap tests of phylogeny in GA (A) and WI (B) WTD using the neighbor-joining method. Color shading differentiates clades supported by bootstrap values greater than 60%. The scale bar represents 0.2 substitutions per nucleotide site for each tree.

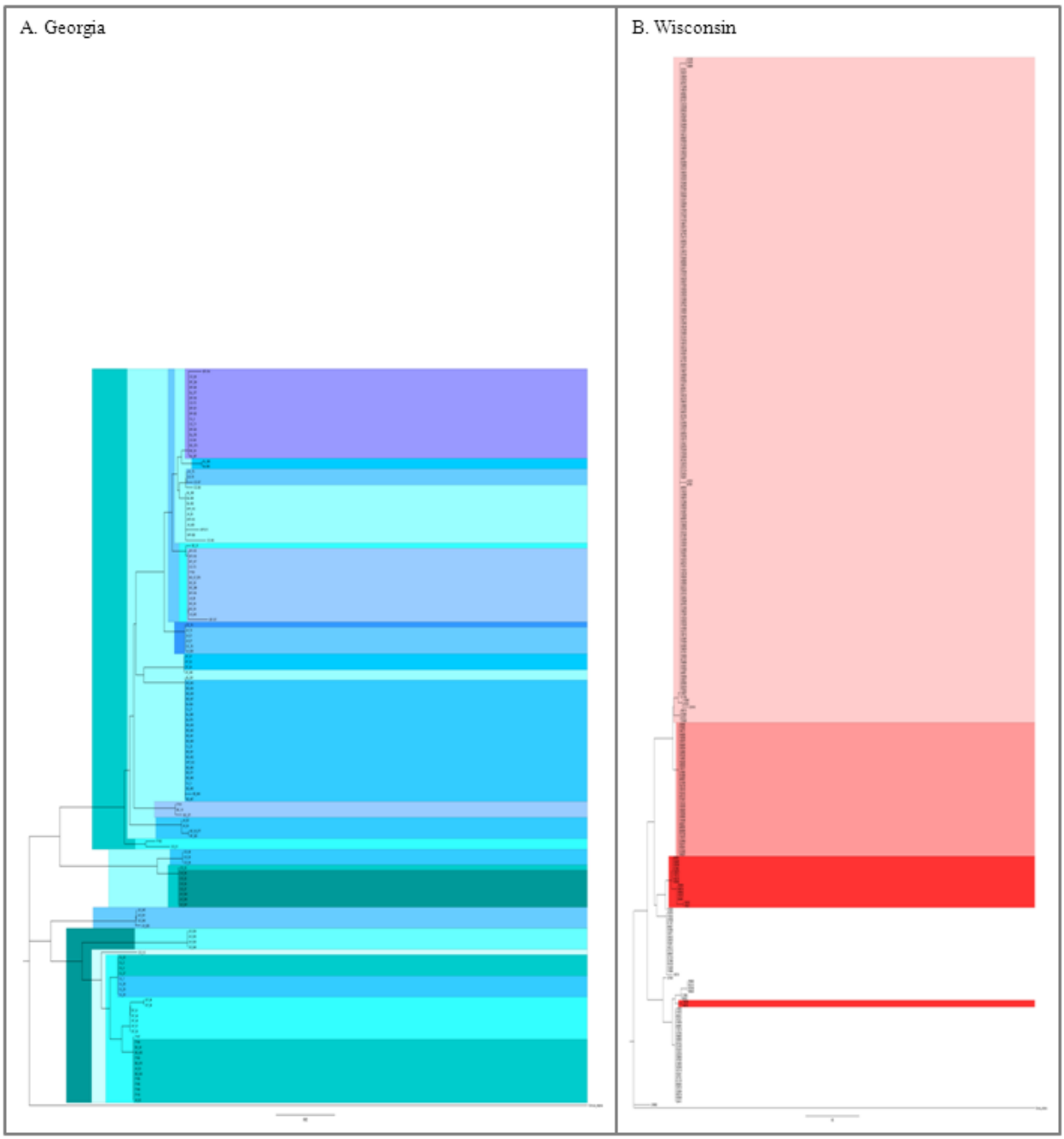
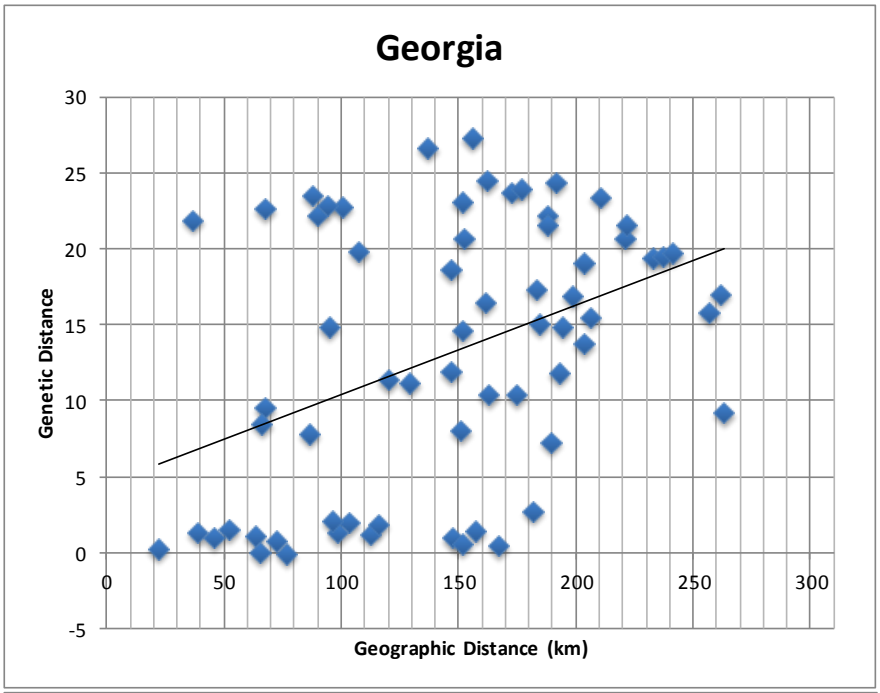


Figure 4.3. Genetic distance by geographic distance. Genetic distances (corrected average pairwise differences) between sampling sites for each state are plotted across geographic distances between sites, with calculated regression lines.

A



B

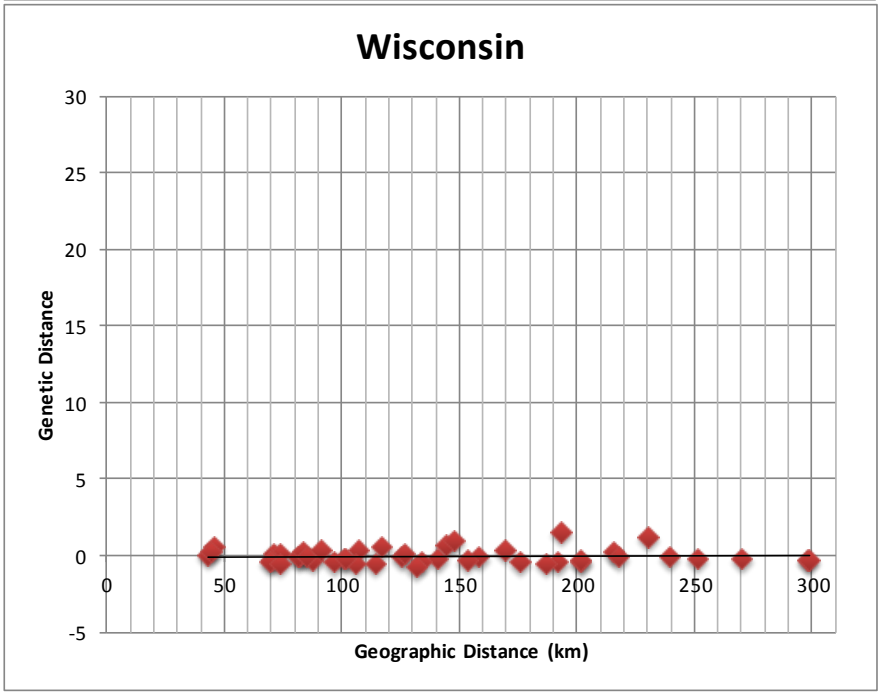
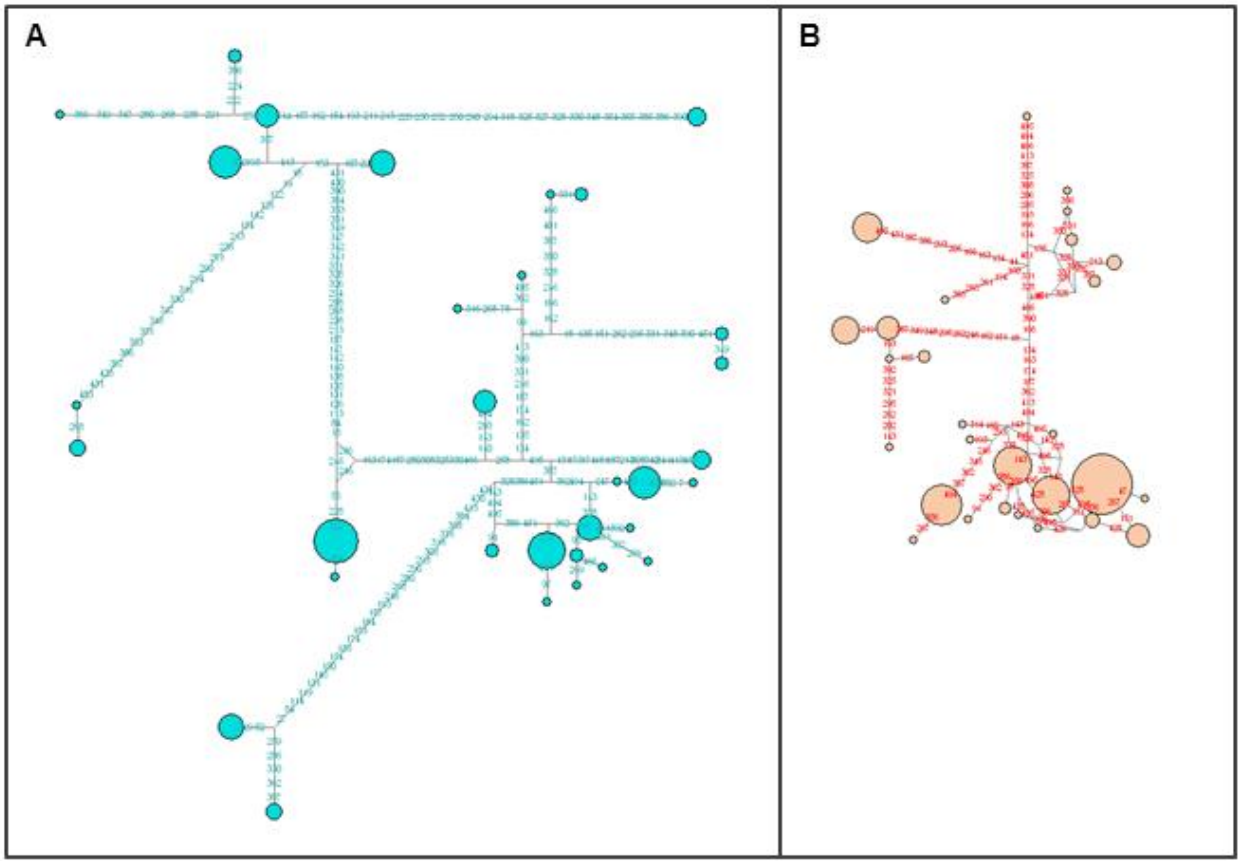


Figure 4.4. Median-joining networks of mtDNA CR haplotypes in GA and WI white-tailed deer. Shaded circles represent distinct haplotypes, with radii proportionate to haplotype frequency across all populations in GA (A) and WI (B). Numbers along network lines represent theoretical mutated positions intermediate between haplotypes.



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CHAPTER 5

SUMMARY AND CONCLUSIONS

Hemorrhagic disease (HD) remains the most significant infectious disease of white-tailed deer (WTD) in the southeastern United States. Disease occurrence has been notably non-uniform nationwide, and in Georgia (GA), geographic variation in HD mortality has demonstrated a consistent spatial pattern (Figure 3.1). Previous studies have implicated host genetic factors in HD susceptibility through experimental infection of immunologically naïve animals from known endemic and epizootic areas (Texas and Wisconsin, respectively). In the early to mid-1900s, over 1500 deer were introduced in GA from out of state, including from areas where HD is endemic or occurs as sporadic severe outbreaks. This study sought to determine whether innate host factors could be a significant influence on HD clinical outcome in GA WTD by characterizing GA population genetic structure, evaluating variability at a major histocompatibility (MHC) locus, and determining the influence of introduced genetics during restocking initiatives of the last century.

In order for host genetics to be a significant factor in spatial differences in HD mortality, there must be sufficient regional genetic differences between areas where deer are apparently resistant to the disease and areas apparently susceptible. Compiled HD reporting data is presented geographically in Figure 3.1, demonstrating relatively fine scale variation, especially in northern Georgia. Through evaluation of mitochondrial D-loop control region (mtDNA CR), we estimated that GA WTD differ significantly on a micro-geographic scale, with a total of 16

sampled wildlife management areas (WMAs) and state parks (SP) being grouped into 8 distinct clusters by pairwise comparisons of nucleotide diversity and haplotype frequency (Chapter 3). Similar comparisons conducted based on microsatellites found a greater degree of intermixing, which is expected in a nuclear marker, taking into account male dispersal. However, even these results suggest strong philopatry in GA WTD with small scale genetic variation. This is especially apparent on Ossabaw Island and in the ridge and valley physiogeographic region in the northwestern corner of the state (Berry College WMA). The Blue Ridge Mountains, piedmont, and coastal plain are also consistently grouped by Bayesian analyses within separate clusters. Observed mortality due to HD is most prevalent in the central piedmont and Blue Ridge Mountains. The clustering we observed in northern GA supports a division between the Blue Ridge Mountains and surrounding areas, which correlates with differences seen in HD mortality. However, individuals in the central piedmont are not significantly different from areas of western piedmont (based on mtDNA and microsatellite analyses) or from areas of eastern piedmont (based on microsatellites). Individuals in the piedmont are significantly different from those found in the coastal plain, according to mtDNA analyses, and are supported by cluster assignments in microsatellite analyses. Overall, we may conclude that there is sufficient fine-scale variation in GA WTD to support regional variations in phenotype, though these variations would likely be minimal, and are not universally consistent with spatial variation in HD mortality. However, as a factor combined with other regional variations, including vector prevalence and competence, virus serotype and virulence, and herd immunity, host genetics could feasibly play a role.

Evaluation of MHC allelic variability and distribution at the MHC Class I heavy chain found variation across GA, but no correlation with HD mortality levels. MHC diversity at this

locus was similar in individuals sampled across the state, though slightly lower on Ossabaw Island, which correlates with low mtDNA diversity at the same site. However, microsatellite variation in these animals suggests sufficient overall genetic diversity consistent with other sampling sites. There were numerous common alleles identified across GA, with no apparent geographic associations between allelic frequencies between sites. Overall we conclude that MHC variation does not vary significantly across the state and is therefore unlikely to contribute to regional variation in HD mortality.

Regional differences in potential foundation genetics across GA due to restocking efforts have the potential to affect modern herds and their phenotype. Source populations for introduced deer included areas where HD viruses are endemic and areas where infrequent epizootics are severe and result in high mortality. In this study, we compared mtDNA haplotypes with haplotypes identified in 21 deer from central Wisconsin (WI). Identical sequences were observed from both states, and in GA, their distribution corresponded with areas historically restocked with WI deer. It is unknown how related mtDNA might be between native GA deer and deer as far north as WI, though it is unlikely that GA haplotypes would be shared with WI deer to the degree we found without the influence of anthropogenic translocations between these sites in recent history. These findings suggest that introductions have had a significant effect on modern deer, though how this might affect HD mortality remains uncertain. Source populations for historical restocking that represent areas where HD outbreaks typically result in significant mortality include WI and the eastern US and Appalachia (NC, KY, and MD). The former group was introduced largely into the western and central coastal plain and piedmont, while the latter group was introduced in the Blue Ridge Mountains and central piedmont, locations with concordant increase in HD mortality reporting (Figure 3.2). Areas restocked with WI WTD that

now share haplotypes with this state correspond with areas of increased HD morbidity reporting, but do not consistently correspond with elevated HD mortality. This may be due to the effects of other factors, including herd immunity.

Further support for the influence of introduced genetics in GA deer is the greater degree of population structure and genetic variability in GA than in WI, which is a state where no restocking program was implemented. Deer in WI underwent significant population declines, though likely not to the degree experienced in GA, and were allowed to recover under protection acts with no introduction of new deer. Our findings suggest a panmictic population of deer in southern WI with limited genetic variability, providing a stark contrast to the high levels of diversity apparent in GA. This difference is most likely attributable to introduced genetics, as the regions studied are roughly similar in size and land cover.

In general, the micro-geographic variations observed in GA, were they to be present in a population with no history of anthropogenic migrations from widely distant areas, would suggest significant barriers to gene flow preventing intermixing over time. This would have resulted in population differentiation through genetic drift, and might eventually lead to the formation of subspecies. However, this scenario would be unlikely in WTD, as they are a large vagile species fully capable of crossing most barriers, natural and man-made. Knowing the history of these populations, however, we have a ready explanation for the high level of spatial variability in GA deer. Introduced genetics from widely distant areas of the United States in the 20th century would have provided a basis for spatial disparities in mtDNA sequence, especially in mtDNA, as females tend to remain within a small home-range, and their female offspring tend to undergo limited dispersal. However, unlike in our previous scenario, where this snapshot of population structuring envisions populations in the process of divergence, the reality is likely quite the

opposite. If our findings depict spatial variation due to introduction history, in the absence of actual restrictions to gene flow, we should expect levels of spatial variation to diminish over time, as slow intermixing of GA populations continues. If host genetics are a significant factor in the spatial pattern of HD mortality in GA, we should expect to see variation diminish over time, as populations continue to slowly mix, eventually resulting in a panmictic population.