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DIVERSITY AND DISTRIBUTION OF WHITE-TAILED DEER mtDNA LINEAGES IN CHRONIC WASTING DISEASE (CWD) OUTBREAK AREAS IN SOUTHERN WISCONSIN, USA

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy affecting North American cervids. Because it is uniformly fatal, the disease is a major concern in the management of white-tailed deer populations. Management programs to control CWD require improved knowledge of deer interaction, movement, and population connectivity that could influence disease transmission and spread. Genetic methods were employed to evaluate connectivity among populations in the CWD management zone of southern Wisconsin. A 576-base-pair region of the mitochondrial DNA of 359 white-tailed deer from 12 sample populations was analyzed. Fifty-eight variable sites were detected within the sequence, defining 43 haplotypes. While most sample populations displayed similar levels of haplotype diversity, individual haplotypes were clustered on the landscape. Spatial clusters of different haplotypes were apparent in distinct ecoregions surrounding CWD outbreak areas. The spatial distribution of mtDNA haplotypes suggests that clustering of the deer matrilineal groups and population connectivity are associated with broad-scale geographic landscape features. These landscape characteristics may also influence the contact rates between groups and therefore the potential spread of CWD; this may be especially true of local disease spread between female social groups. Our results suggest that optimal CWD management needs to be tailored to fit gender-specific dispersal behaviors and regional differences in deer population connectivity. This information will help wildlife managers design surveillance and monitoring efforts based on population interactions and potential deer movement among CWD-affected and unaffected areas.

Chronic wasting disease (CWD) is a transmissible spongiform encephelopathy (TSE) that affects white-tailed deer (*Odocoileus virginianus*), and other North American members of the cervid family including mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*), and moose (*Alces alces*) (Baeten et al. 2007; Williams 2005). Chronic wasting disease is related to other TSEs, including Creuzfeldt–Jakob disease (CJD) in humans, bovine spongiform encephelopathy (BSE; mad cow disease) in cattle, and scrapie in sheep. Progression of CWD, like other TSEs, is characterized by lengthy incubation, lack of immune response, infection of lymphatic and nervous tissue, spongiform lesions in the brain with associated

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neuronal degeneration, and clinical signs associated with advanced brain infection (Centers for Disease Control and Prevention 2006; Williams et al. 2002). CWD has affected free-ranging deer in at least 15 states and two Canadian provinces and has had considerable economic and ecological impact, particularly in Wisconsin (Heberlein 2004).

Chronic wasting disease has been a primary wildlife management concern in southern Wisconsin, where affected white-tailed deer may be in contact with humans and livestock. While there is limited risk of transmission to humans (World Health Organization 2007) or livestock (Hamir et al. 2007), CWD's close relationship to other disease like BSEs has given many cause for concern (Conner et al. 2008). Both the hunting public and natural resource agencies are concerned about future deer populations in the face of CWD, which might adversely impact populations with high rates of infection. Where the disease has been long established, high prevalence rates led to declines in the deer population (Miller et al. 2008). Further, deer hunting provides substantial input into the economy of Wisconsin (nearly $1.4 billion in 2006; Wisconsin Department of Natural Resources 2010). Surveys showed that if CWD prevalence reaches 50%, almost half of hunters would stop hunting, which could devastate some rural communities that rely on hunting revenue (Wisconsin Department of Natural Resources 2010). Therefore, it is critical to understand the disease transmission dynamics and spread so that management agencies like the Wisconsin Department of Natural Resources (WDNR) can develop strategies to reduce or prevent CWD spread across the region.

Chronic wasting disease management presents numerous challenges: (1) Currently there are no treatments, (2) identifying infected individuals is difficult, and (3) little is known regarding the relative importance of transmission via direct contact versus environmental contamination (Mathiason et al. 2009). Surveillance for new disease areas (Samuel et al. 2003) and culling programs in affected areas are the primary means of disease management (Williams et al. 2002; Wisconsin Department of Natural Resources 2009), which would benefit from improved understanding of deer social and behavioral factors affecting disease dynamics (Altizer et al. 2003; Cross et al. 2008; Schauber et al 2007). Both the interactions within social groups and the movements between groups affects disease transmission and spread (Cross et al. 2008); thus, it is particularly important to understand how animals interact at the local scale as well as the landscape scale. For example, dispersal or seasonal movement by cervids increases infectious contacts between animals and spreads infectious prions to new regions (Conner et al. 2008). Thus, understanding connectivity and dispersal patterns among the deer populations might provide an important foundation for predicting and mitigating disease spread across the landscape. While much attention has been paid to the dispersal of male deer (Diefenbach et al. 2008; Long et al. 2005; Hölzenbein and Marchinton 1992), less is known regarding the movements and population connectivity of more philopatric females (Nelson and Mech 1992).

In this study population genetic techniques, which can be particularly useful in elucidating patterns of connectivity and contact between populations, were employed. Though other strategies (e.g., tagging or telemetry) are also useful, genetic analysis can efficiently achieve large sample sizes over broad spatial extents to provide a farther reaching picture of dispersal patterns and population structure (Deyoung and Honeycutt 2005). Mitochondrial DNA (mtDNA) patterns, in particular, are useful in depicting landscape-scale population structure. DNA located in the mitochondria is maternally inherited and it is haploid, which negates the effects of recombination, leaving a clearer view of matrilineal gene flow (Avise et al. 1987). The geographic distribution of mtDNA genetic variation may be used to identify broad patterns of population connectivity (Deyoung and Honeycutt 2005). In mammalian species exhibiting female philopatry, mtDNA patterns

are often more spatially structured than those based on nuclear markers like microsatellites (Avise 1994). While mtDNA may be limited for detecting fine scale patterns within the current-generation time scale, it might provide extra sensitivity for detecting landscape factors affecting gene flow at the broad scale (Avise et al. 1987; Piertney et al. 2000; Purdue et al 2006).

In deer populations, female philopatry forms an important basis of population social structure, with groups of related females comprising the basic social unit (Hawkins and Klimstra 1970). As such, animal-to-animal contact is likely to be elevated within social groups, creating potential for localized amplification of disease prevalence (Grear et al. 2010). While males may be responsible for much of the contact between populations, females may be the primary vectors of CWD transmission within social groups, and between populations in close proximity (Cross et al. 2008; Cullingham et al. 2010; Grear et al. 2010). Thus, describing the matrilineal genetic structure might be especially informative for understanding local disease dynamics. Matrilineal structure has been linked to dynamics of other diseases (Blanchong et al. 2007).

This study was motivated by the need to (1) better understand animal movements and population connectivity across the landscape and (2) provide a foundation for CWD management and containment strategies. mtDNA sequencing and spatial analysis were used to investigate population genetic patterns relative to the CWD-infected areas of southern Wisconsin. Our objectives were to (1) determine the diversity and distribution of mtDNA genetic lineages within and around the CWD management zone, (2) describe the degree of population differentiation to understand potential contact via deer dispersal, and (3) analyze genetic patterns to understand how geographic features shape population connectivity in the southern Wisconsin landscape. Through this investigation it is hoped to better understand how population connectivity varies across the landscape and might influence the ability of CWD to spread between populations.

METHODS

Study Area and Sample Collection

Our study area was the Chronic Wasting Disease Management Zone (CWD-MZ) in southern Wisconsin. The CWD-MZ is about 9000 square miles surrounding the two core outbreaks (Joly et al. 2006) by about 40 miles and encompasses more than 12 counties (approximate geographical extent from 43°43′ N, 90°54′ W to 42°30′ N, 87°57′ W; Figure 1). The western core (WCWD), primarily in Dane and Iowa counties of south-central Wisconsin, was detected in 2002 and exhibits the highest local prevalence of CWD (>6% in adults in some townships). The eastern core (ECWD), primarily in Rock and Walworth counties, and extending into Winnebago and Boone counties in Illinois, was detected the following year. Sporadic disease cases, or sparks, were also detected in areas away from the infection cores. Epidemic patterns suggest that the disease has been present in the region for at least 20 yr (Wasserberg et al. 2009).

The CWD-MZ is comprised primarily of three ecoregions: the Western Coulee and Ridge and Southwest Savanna ecosystems in the west, and the Southeast Glacial Plains in the east (Omernik 1987). A small portion of the Central Sand Hills enters the study area at the north. The WCWD core lies in the Western Coulee and Ridge ecoregions, characterized by variable topography with eroded valleys. The area is primarily hardwood forests with small crop parcels in flatter valleys (Wisconsin Department of Natural Resources in preparation). The Western Coulee and Ridge region hosts some of the densest deer populations in Wisconsin; some areas have >99 deer per 2.6-km^2 section of deer habitat, with deer habitat comprising 55–77% of the landscape in this area (Rolley 2007). The Southwest Savanna has more gently rolling topography with scattered forests. More than three-quarters of this region is in agricultural production with numerous small natural prairie remnants (Wisconsin Department of Natural Resources in preparation). The ECWD core occurs within the

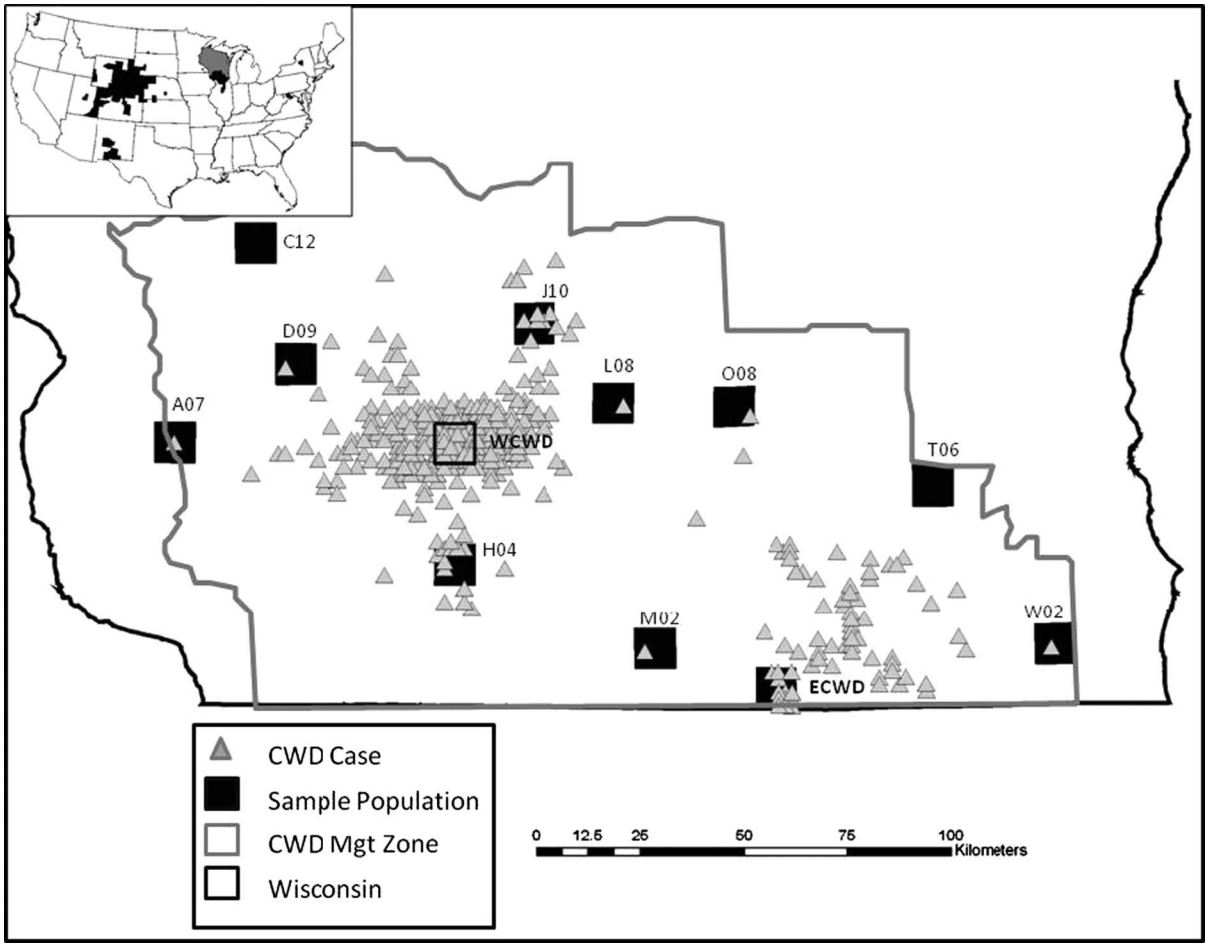

FIGURE 1. Study area with townships sampled for white-tailed deer, as well as CWD cases in southern Wisconsin, 2002–2008. The inset map depicts the distribution of CWD in free-ranging cervids throughout the United States, withWisconsin shaded in gray. See Table 1 for information on sample sizes in each sample population.

Southeastern Glacial Plains. This area, once heavily glaciated, consists of open plains and glacial moraines. In recent times nearly all of this plains region has been shaped by agriculture and residential settlements; only small fragments of forest and native grasslands remain (Wisconsin Department of Natural Resources in preparation). Deer densities in the Southeast Glacial Plains are lower than in the west, with <66 deer per 2.6-km^2 section, and deer habitat is more sparsely distributed throughout this ecoregion (making up just 17–40% of the area) (Rolley 2007).

Genetic samples were obtained from 363 deer from populations represented by 12 separate townships (the average township had 30 individuals, but one consisted of only 5 deer and was excluded from spatial analyses; Table 1). Our sample included 55 deer from the WCWD sequenced for a previous study (Grear et al. 2010) and 11 newly sampled townships. Lymph nodes for genotyping were collected from harvested deer by WDNR staff between 2002 and 2008 as part of their CWD surveillance program. When the samples were collected the hunters indicated the harvest location on a map, specifying to the public land survey system (PLSS) section (2.6 km^2). To the extent possible, samples were selected distributed over the 36 sections of each sampled township (an average of 20 sections was sampled per township, with a minimum of 15 sections, excepting L08). Considering the generally small home ranges of

TABLE 1. Measures of Genetic Diversity in White-Tailed Deer From Southern Wisconsin, 2002–2008

Population	n	*H*	± SD	π	± SD
Ao7	28	0.974	±0.018	0.019	±0.010
C12	31	0.951	±0.021	0.019	±0.010
D09	43	0.929	±0.031	0.012	±0.006
ECWD	33	0.980	±0.015	0.022	±0.011
H04	34	0.959	±0.022	0.015	±0.008
J10	34	0.947	±0.021	0.014	±0.007
L08	5	0.900	±0.161	0.003	±0.003
M02	21	0.962	±0.030	0.013	±0.007
O08	20	0.953	±0.036	0.016	±0.008
T06	36	0.875	±0.038	0.016	±0.008
W02	23	0.980	±0.020	0.021	±0.011
WCWD	55	0.457	±0.085	0.008	±0.004
Total	363	0.959	±0.005	0.016	±0.008

Note: Sample size (n) and nucleotide (π) and haplotype (*H*) diversities of each sample population included in the study. Values were calculated using Arlequin v3.5 based on a 576-base-pair region of the mtDNA control region.

adult female deer (<1 km^2), our samples likely represented many different female matrilineal groups. In addition to harvest location, the age and gender of deer were recorded. The age was determined by examining the tooth wear of the deer (Severinghaus 1949). Our sampling efforts focused on adult females to maximize the detection of population structure; however, where samples were sparse, males were also included. The sample contained 289 female deer and 74 males. In all of the townships the number of female deer was greater than male deer, ranging from almost 1 to 1, to a high of nearly 6 to 1. All deer were more than 1.5 years old and would thus be expected to have established their adult range.

DNA Extraction and Sequencing

Genomic DNA was extracted and purified according to the protocol outlined by the manufacturer, using the Qiagen DNeasy extraction kit (Qiagen, Germantown, MD). After the DNA was isolated it was quantified using ultraviolet (UV) spectroscopy and diluted to a standard concentration of 20–50 ng/μl. Subsequently a 576-base-pair sequence of the mtDNA control region was amplified using polymerase chain reaction (PCR) (primer set F1, 5′- TCT CCC TAA GAC TCA AGG AAG -3′, and R1, 5′-GTC ATT AGT CCA TCG AGA TGT C -3′, developed by Miyamoto et al. [1990]; Genbank Accession ODOMTFVLA). A 10-μl PCR mix was used containing 5 μl Qiagen multiplex mix, 1 μ*M* concentration of primer mix, and approximately 20 ng DNA sample. The amplification was run at 94°C for 30 s, 56°C for 90 s, and 72°C for 60 s and repeated for 40 cycles. The product was sequenced in the forward direction (using primer F1 5′- TCT CCC TAA GAC TCA AGG AAG -3′) with the Big Dye di-deoxy sequencing protocol (Applied Biosystems, Carlsbad, CA) at the University of Wisconsin (UW)–Madison Biotech Center. The program Sequencher v4.1 (Gene Codes Corporation, Ann Arbor, MI) was used to view chromatographs of the haplotype sequences and manually correct any errors in nucleotide base determination to generate haplotype assignments.

Genetic Diversity

Basic statistics of haplotype and nucleotide diversity (Nei 1987) were reported for the total sample set and for each sampled population (calculated in Arliquin 3.5; Excoffier, Laval, and Schneider 2005). An analysis of molecular variance (AMOVA; Excoffier, Smouse, and Quattro 1992) was employed to describe basic population genetic structure according to the partitioning of haplotype diversity at levels of regions (samples around the ECWD vs. those around the WCWD), sampled townships, and individuals within townships. The values of Fst are also presented, indicating the degree of genetic differentiation between each pair of townships (AMOVA and Fst calculated using GenAlEx v6; Peakall and Smouse 2006).

A network diagram was constructed to display the phylogenetic relationship between haplotypes in the southern Wisconsin deer population (Figure 2). A median-joining network was created using the program Network v4.6 (Fluxus Technology, Inc., Suffolk, England). The network is built by finding the most parsimonious tree that connects sequences that minimize total phylogenetic branch length

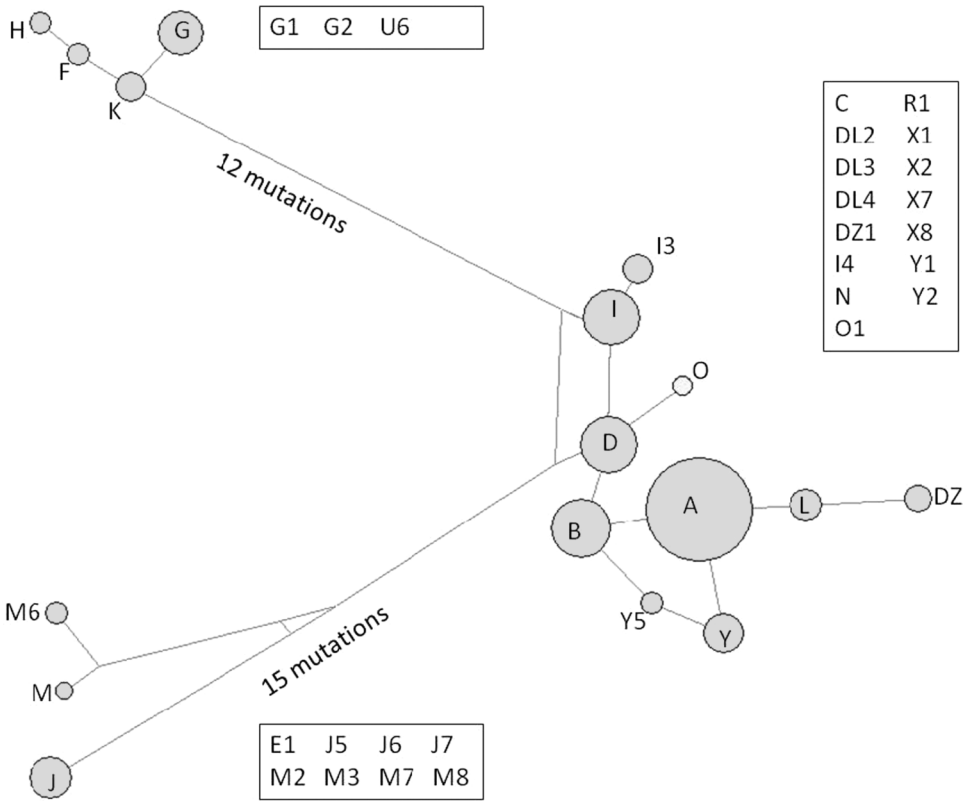

FIGURE 2. Phylogenetic network depicts the relationship between mtDNA haplotypes observed in white-tailed deer from southern Wisconsin. Node sizes are proportional to the frequency of the given haplotype, and line length is proportional to the number of base pair differences between the haplotypes. To simplify the image, rare haplotypes (occurring in three or fewer individuals) are listed in boxes adjacent to the clade containing those haplotypes. The diagram was generated in Network v4.6 based on a 576-base-pair region of the mtDNA control region.

(based on algorithms of Kruskal 1956; implemented by Bandelt et al. 1999). This method is well suited for large sample sizes with no recombination, and infrequent ambiguous states (Bandelt et al. 1999).

Spatial Genetic Analysis

In addition the hierarchical description of genetic structure in the AMOVA, a geostatistical approach was used to explicitly consider patterns of haplotype diversity on the landscape. Haplotypes were mapped in Arc GIS 9.2 (ESRI 2006) to display the geographic distribution of the haplotypes across the CWD-MZ. A two-step approach was used to quantify the spatial arrangement of the haplotypes. Initially, an Allele Aggregation Index (AAI) (using the Alleles In Space software; Miller 2005) was calculated. The AAI provides a test for nonrandom patterns of haplotype occurrence across space, similar to common aggregation indexes employed in other areas of ecology (Clark and Evans 1954; Pommerening 2002). The index value is 1 when individuals of each haplotype are randomly distributed among sample sites, and less than 1 when like haplotypes are aggregated (Pielou 1977). Test statistics were generated separately for each haplotype as well as a global statistic for the multi-allelic pattern.

Local autocorrelation statistics were also calculated to determine whether each haplotype was distributed randomly across the landscape, or clustered in local regions of the CWD-MZ. Moran's I (Moran 1950) has been widely applied as a global measure of genetic analyses (Epperson 2003; Hardy and Vekemans 1999). In contrast, Moran's

LISA (local indicator of spatial association) computes joint covariation of neighboring localities using the average haplotype frequency within a locally defined neighborhood. The LISA assesses the spatial pattern at each sample township against an expected value generated for each location based on the local neighborhood (Anselin 1995; Fortin and Dale 2005). The LISA test offers the advantage that values can be mapped to identify position, size, shape, and layout of spatial genetic patterns (Fortin and Dale 2005). LISA analysis identified areas where allele frequencies are more similar than expected by chance, signifying a spatially restricted distribution (cluster) of a specific haplotype. The Moran's LISA analysis was implemented based on mtDNA frequencies in sampled townships (implemented in the geostatistical program Geoda v 0.9.5-i; Anselin 2004). Spatial clustering only of haplotypes that occurred in at least five individuals (avoiding rare haplotypes with little power to determine patterns) was assessed. The local neighborhoods were defined to include all population pairs within 250 km, which effectively encompassed the entire study area, making the LISA simply a mapped breakdown of the CWD-MZ spatial pattern. Significance of local clusters was calculated using 999 randomizations in Geoda. Finally, clusters of similar haplotype frequencies were identified by joining them with lines to indicate the minimum convex polygon spanned by each cluster (Figure 3).

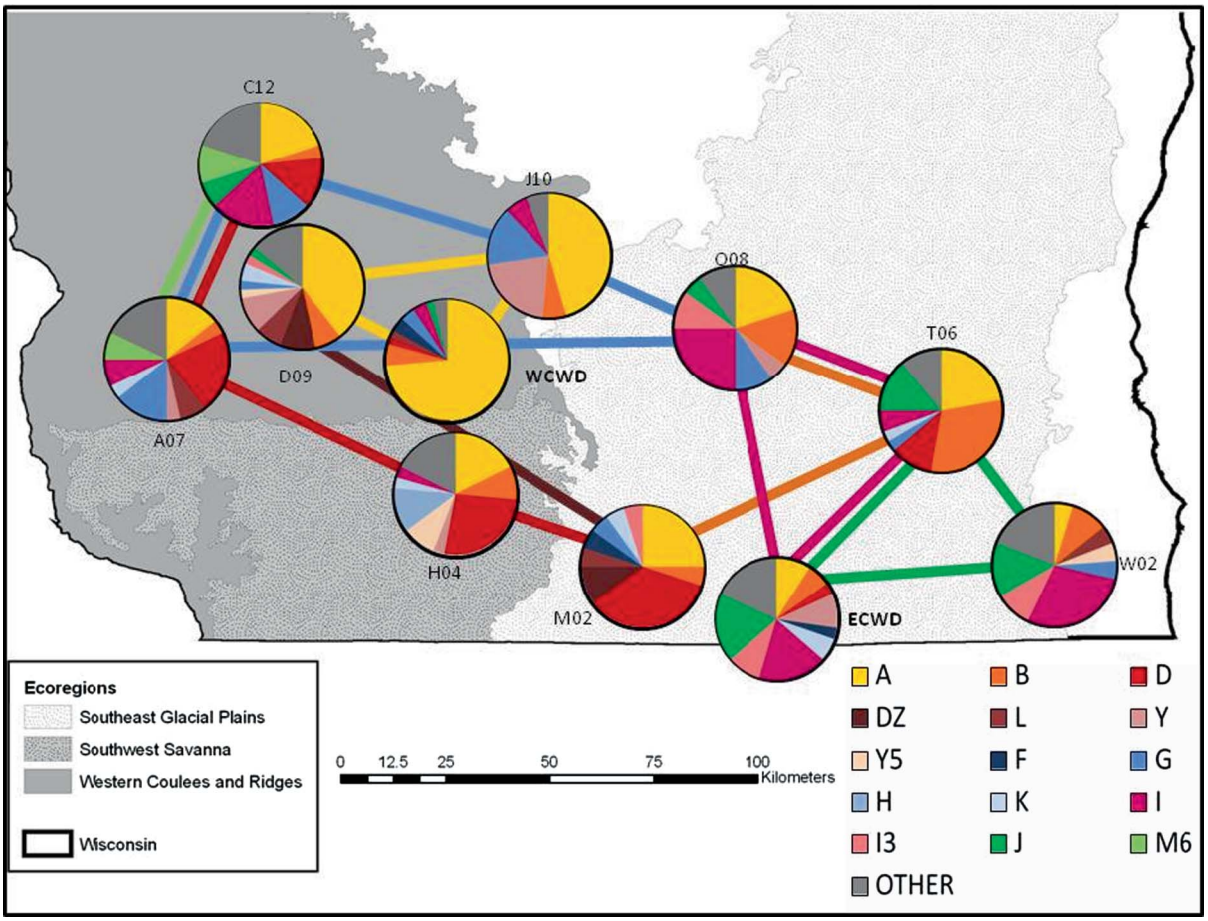

FIGURE 3. Pie charts illustrate the distribution of mtDNA haplotypes throughout white-tailed deer populations in the CWD-affected region of southern Wisconsin. Lines connect populations that share spatially clustered haplotypes (based on LISA statistic, coded by color matching the haplotype designations in pie charts). Pie graphs are centered over sampled townships depicted in Figure 1; sample size information is available in Table 1 (color figure available online).

RESULTS

Genetic Diversity

High sequence diversity was detected in the southern Wisconsin white-tailed deer population. After realigning and trimming the sequences, 58 variable sites were identified, making up 43 haplotypes in a 576-bp fragment. In total, 55 transition sites were identified along with 6 transversion sites. Haplotype diversity for the total population was 0.959 ± 0.005, and the nucleotide diversity was 0.016 ± 0.008 (Table 1). Measures of diversity were similar for most of the 12 sampled townships (ranging 0.900 to 0.980). However, the WCWD township had low haplotype ($H = 0.457$) and nucleotide diversity ($\pi = 0.008$) compared to other townships. Township L08, with five samples, also had low nucleotide diversity compared to the other townships ($\pi = 0.003$). The range for nucleotide diversity for other townships was 0.122 to 0.022 (Table 1).

Haplotypes separated into three clades of closely related lineages (Figure 2). The most populated clade contained several of the most frequent haplotypes, A, B, D, I, and L, which were separated by only a few base differences. The second clade (F, G, H, K) was separated by 12 base differences, and the third (J, M) was 15 base mutations from the primary clade (Figure 2).

Moderate Fst values indicated low levels of differentiation between most study populations (Table 2). All comparison to the WCWD exhibited elevated Fst values, given the high frequency of haplotype A in the WCWD population. Differentiation was higher among western populations than those in the eastern portion of the study landscape; Fst measures between townships averaged 0.045 in the eastern portion of the study area and 0.051 in the west, if high values from the WCWD are excluded (0.1 when including the WCWD). Fst values averaged 0.057 when comparing populations across the eastern and western regions. The AMOVA further indicated partitioning of genetic variability, first at the level of regions (3% of the total variation), and also between townships (7% of the total variation). In contrast, microsatellite data (Robinson unpublished data) had Fst values averaging 0.021 between these populations, and an AMOVA based on the biparentally inherited microsatellites showed only 1% of variation occurring between regions and 3% between populations, with the remainder allocated between individuals.

Spatial Genetic Analysis

Our spatial analysis revealed a clustered distribution of haplotypes across the study

TABLE 2. Pair-Wise Fst Measurements Between Sample Populations of White-Tailed Deer From Southern Wisconsin, 2002–2008

	Western half						Eastern half			
	A07	C12	D09	H04	J10	WCWD	ECWD	M02	O08	T06
Western half										
C12	0.000									
D09	0.064	0.059								
H04	0.009	0.036	0.074							
J10	0.078	0.068	0.012	0.112						
WCWD	0.295	0.243	0.086	0.270	0.099					
Eastern half										
ECWD	0.043	0.021	0.081	0.066	0.107	0.336				
M02	0.000	0.027	0.060	0.000	0.106	0.260	0.081			
O08	0.030	0.004	0.052	0.068	0.053	0.254	0.000	0.077		
T06	0.050	0.042	0.063	0.047	0.099	0.232	0.046	0.057	0.025	
W02	0.048	0.021	0.113	0.087	0.144	0.412	0.000	0.105	0.000	0.059

Note: Values were calculated using GenAlex v6.1 based on a 576-base-pair region of the mtDNA control region.

landscape. The global test of the Allele Association Index indicated that in general, common haplotypes were spatially clustered (Table 3). Several specific haplotypes exhibited strong spatial clustering (A, DZ, H, I, and M6, Table 3). Additionally, the LISA statistic identified significant local autocorrelation for many haplotypes (marked with an asterisk

TABLE 3. Distribution of the 43 Haplotypes Identified in White-Tailed Deer From a 576-Base-Pair Region of the mtDNA Control Region

Haplotype	A07	C12	D09	W-CWDcore	H04	J10	L08	M02	E-CWDcore	O08	T06	W02	Total N	AAI	*p* Value
Spatial analysis performed															
Over all haplotypes														0.703	0.000**
A	4	6	17*	39*	6	15*	3	5	3	4	8	1	111	0.240	0.038*
B	1	1	3	3	3	2	1	1	2	3*	11**	2	33	0.435	0.850
D	6**	4**	0	1	9**	0	0	7**	1	0	4**	0	32	0.242	0.081
DZ	0	0	4*	0	0	0	1	2*	0	0	0	0	7	0.407	0.047*
G	4*	3**	1	2	0	5**	0	1	0	2**	1	1	20	0.669	0.856
I	2	5	0	2	0	2	0	0	6**	5*	2	6*	30	0.207	0.017*
I3	0	0	1	0	1	0	0	1	3	2	0	2	10	0.681	0.318
J	0	2	1	1	0	0	0	0	6**	1	5**	3**	19	0.514	0.409
K	1	0	2	0	1	0	0	1	2	0	1	0	8	0.822	0.428
L	2	0	3	1	0	0	0	1	0	0	0	1	8	0.965	0.609
M6	2*	3**	0	0	0	0	0	0	0	0	0	0	5	0.115	0.005**
Y	1	0	4	0	1	7	0	0	3	1	0	0	17	0.508	0.330
Y5	0	0	1	0	3**	0	0	0	0	0	0	1	5	1.178	0.644
No spatial analysis															
C	0	0	0	2	0	0	0	0	0	0	0	0	2		
DL2	1	0	0	0	0	0	0	0	0	0	0	0	1		
DL3	0	1	0	0	0	1	0	1	0	0	0	1	4		
DL4	0	0	0	0	2	0	0	0	1	0	0	0	3		
DZ1	0	0	0	0	1	0	0	0	0	0	0	0	1		
E1	0	0	0	0	0	0	0	0	0	1	0	1	2		
F	0	0	0	2	0	0	0	1	1	0	0	0	4		
G1	0	0	0	0	0	0	0	0	0	0	1	0	1		
G2	0	0	0	0	0	1	0	0	0	0	0	0	1		
H	0	0	0	0	4	0	0	0	0	0	0	0	4		
J4	0	0	0	0	0	0	0	0	1	1	0	0	2		
J5	0	0	0	0	0	0	0	0	1	0	0	0	1		
J6	1	0	0	0	0	0	0	0	0	0	0	0	1		
J7	0	0	0	0	0	0	0	0	0	0	1	0	1		
M	0	1	0	0	0	0	0	0	1	0	0	0	2		
M2	1	0	0	0	2	0	0	0	0	0	0	0	3		
M3	0	0	0	0	0	0	0	0	2	0	0	1	3		
M7	0	0	0	0	0	0	0	0	0	0	0	2	2		
M8	0	0	1	0	0	0	0	0	0	0	0	0	1		
N	0	0	0	0	0	0	0	0	0	0	0	1	1		
O	0	0	3	0	0	0	0	0	0	0	0	0	3		
O1	0	0	1	0	0	0	0	0	0	0	0	0	1		
R1	0	3	0	0	0	0	0	0	0	0	0	0	3		
U6	0	0	0	0	0	0	0	0	0	0	1	0	1		
X1	1	0	0	0	0	0	0	0	0	0	0	0	1		
X2	0	2	0	0	0	0	0	0	0	0	0	0	2		
X7	0	0	0	0	1	0	0	0	0	0	0	0	1		
X8	0	0	1	0	0	0	0	0	0	0	0	0	1		
Y1	1	0	0	0	0	1	0	0	0	0	0	0	2		
Y2	0	0	0	0	0	0	0	0	0	0	1	0	1		

Note: Sample populations listed across the top show the distribution of haplotypes within southern Wisconsin, 2002–2008. Asterisks indicate the townships in which specific haplotypes were spatially clustered; *indicates p value $< .05$ in the LISA test, **for p value $< .001$. (Note: after Bonferroni correction for multiple tests over 12 populations, the critical p value of interest is .004.) The AAI column gives the allelic aggregation index, with p values marked similarly to identify significantly clustered haplotypes. For rare haplotypes the bottom portion of the table reports only their occurrence; spatial analysis was not conducted due to insufficient number of samples with these haplotypes. Township designations can be matched with Figure 3 to identify the geographic distribution of the haplotypes.

in Table 3). The LISA analysis was employed to identify local concentrations of haplotypes (Figure 3). Significant local clusters were found in some haplotypes without significant AAI results. Because LISA statistics were calculated on the allele frequencies per sample township, rather than the point locations of each individual, it was likely more sensitive to the clustering of haplotypes.

More common haplotypes were clustered throughout the study area with distinct haplotype clusters in the Western Coulee and Ridge and Southeast Glacial Plains ecoregions (Figure 3). For example, haplotypes A, G, and M6 formed significant clusters concentrated in the western portion of the study area, whereas B, I, and J were significantly clustered in the eastern region (Figure 3, significance given in Table 3). In general, townships containing rare haplotypes (frequencies < 0.1) tended to exhibit local clusters of high frequency in a landscape generally lacking the haplotypes; for example Y5 or DZ. Because these haplotypes were rare one can conclude little about their spatial arrangement because the locations of these haplotypes might be influenced by the probability of detecting rare haplotypes in the sampled population.

DISCUSSION

Genetic Diversity

A high level of haplotype diversity was found and considerable mixing of haplotypes occurred across the CWD management zone in southern Wisconsin. Measures of high haplotype diversity and lower nucleotide diversity observed in our study were similar to other studies of cervids which indicated that minor differences in nucleotide structure contribute to the formation of several haplotypes (Cullingham et al. 2010; Hartl et al. 2005). The overall level of haplotype diversity and phylogenetic pattern were likely shaped by ancient processes of landscape change and shifts in species ranges (Cronin 1991). As the last glaciers receded after the Pleistocene ice age and deer moved into newly opening habitat, Wisconsin populations were probably founded by a few lineages constituting the major clades detected in our study, with subsequent divergence over the past 10,000 years forming the observed diversity of haplotypes.

More recent demographic history and harvest pressures may continue to influence the fine-scale spatial patterns of existing haplotypes. Deer in the Midwest where overharvested and habitat was converted to agriculture around the turn of the 20th century, dramatically reducing the abundance and distribution of deer (Bersing 1966). This population decline may have led to local extirpation of some haplotypes (Allendorf et al. 2008). As the deer population rebounded, temporal changes in the population may have influenced the local spatial structure of mtDNA lineages (Scribner et al 1997). Hunting pressure might also influence the level of haplotype mixing by influencing the distance that deer disperse (Comer et al. 2005) and the breakup and dispersion of matrilineal social groups, increasing range overlap and contact between deer from unrelated groups (Williams et al. 2008).

An interesting exception to the high level of haplotype diversity and spatial mixing was found in the WCWD township with relatively low haplotype diversity, predominantly composed of the A haplotype. This raises questions linking genetic diversity and disease (DeWoody 2005; Gillespie 1975); however, mtDNA diversity is much higher in the ECWD, indicating that CWD is not associated with a lack of mtDNA diversity. Further, the difference in genetic diversity between these two infected areas also indicates that establishment of CWD was not produced by populations that are immune-compromised by poor genetic diversity. There are several alternate explanations to consider. First, WCWD haplotypes were originally generated in a different laboratory; however, 24 of the 55 samples were retyped in our laboratory and no discrepancies were found. It is also possible that spatial clustering in sample collection could bias the observed genetic patterns; however, individuals were sampled from 23 sections within the WCWD, ruling out the

possibility that our sample was restricted to a few social groups. Samples in the WCWD were collected earlier than other samples, creating the potential for temporal bias in our genetic patterns. This is particularly concerning because population reduction efforts during CWD management may have decreased genetic diversity; however, the WCWD was sampled almost entirely in 2002, before population reduction took place. Other townships in our study area were not subject to CWD management regimes, so one might expect little temporal effect for areas sampled from 2004 to 2007. Perhaps the most likely explanation for low genetic diversity within the WCWD is historic isolation or founder effects during the time of population recovery (Moritz 1994). Landscape genetics investigations showed that the rivers and highways around the WCWD may impede local gene flow, lending support to the idea of isolation of this population (Blanchong et al. 2008). Additional research is merited to further examine the depression of mtDNA diversity in the WCWD where CWD has become established.

Spatial Genetic Analysis

Our observations of genetic diversiy and differentiation across the Wisconsin landscape indicate generally strong connectivity between populations. Relatively low Fst values also suggested that most populations were connected through gene flow. However, genetic mixing was not random and ecological features appear to shape the variation in gene flow across the region. Haplotypes were observed from three divergent clades geographically distributed across the study area. Our results showed more intermixing of haplotypes than was noted in deer populations in other parts of the species range as evidenced by Purdue et al. (2000) who found little spatial overlap of haplotypes in South Carolina. However, other investigations in similar Midwestern environments yielded comparable levels of high haplotype diversity and substantial spatial mixing of haplotypes (J. Blanchong personal communication).

Within the pattern of generally high population connectivity, spatial clustering of mtDNA haplotypes, as shown by the AAI and LISA analysis, indicated that female gene flow was localized across our study landscape, as one might expect from mammal species exhibiting female philopatry and matrilineal social structure (Deyoung and Honeycutt 2005; Miller et al. 2010; Purdue et al. 2006). The LISA statistic allowed us to extend the more typical global autocorrelation analysis and identify specific ecoregions where haplotypes were clustered, suggesting that broad-scale population structure was shaped by landscape characteristics. The AMOVA offered additional statistical support that regional differences played a role in the distribution of haplotypes. It is possible that regional movement patterns and natal habitat fidelity influence the distribution of haplotypes throughout the area. Other studies found regional habitat distinctions to influence philopatry in deer (Pease et al. 2009), as well as other far-ranging species (Sacks et al. 2005).

The genetic patterns detected are concordant with previous research demonstrating the impacts of habitat quality and arrangement on deer dispersal (Diefenbach et al. 2008; Long et al. 2005). Telemetry research showed that migration and dispersal are more common and longer ranging in areas with sparse or less permanent forest cover—even among generally philopatric females (Nixon et al. 2007; 2008). These patterns corroborate our finding of differences between the forested western ecoregion and the open plains habitat in the east, with generally lower levels of differentiation among populations in the Southeast Glacial Plains. This might also help explain the more clustered pattern seen around western populations, particularly the WCWD. The high levels of female movement in open habitat likely influenced the amount of geographic mixing and distribution of haplotypes that was observed.

CWD Management Implications

Although the mechanisms responsible for CWD spread are uncertain, dispersing deer probably play an important role (Blanchong

et al. 2007). Differences in behavioral and movement patterns between male and female deer may result in differing roles in the spread and propagation of CWD. Recent data demonstrated that spatial epidemiological patterns may differ by gender (Heisey et al. 2010). Because male deer are more likely to disperse farther and maintain larger home ranges (Hawkins and Klimstra 1970; Rosenberry et al. 1999), males display the potential to carry disease over greater distances and contribute to jumps in the spatial spread of CWD (Conner et al. 2008; Grear et al. 2006). Females are more philopatric, and likely to engage in close social contact within kin groups, which may elevate the risk for disease transmission between related females (Grear et al. 2010). Thus, understanding the scale and arrangement of female groups might be critical in understanding the potential for gradual propagation of infection and the spread of CWD among overlapping (or adjacent) social units. Our study provides preliminary evidence that landscape factors may influence dispersal and movement of female deer and consequently influence disease spread. In particular, female deer may be more likely to interact within ecoregions than between ecoregions. In future research efforts, it will be important to add information from biparentally inherited nuclear genetic markers (Cullingham et al. 2010; Grear et al. 2010). While the mtDNA patterns provide a good measure of highest potential population genetic structure, it is worth noting that even those populations strongly differentiated in mtDNA signatures may be connected through male-mediated gene flow. This underscores the importance of considering sex-specific behaviors in understanding deer movement and potential contact rates relevant to CWD management.

The regional connectivity of deer lineages identified in our study corresponds not just to ecoregions; but, importantly, to CWD outbreak areas. The clustering of different sets of mtDNA lineages around each core area suggests that female dispersal is localized and contact rates may be highest within similar habitat zones. Thus, disease spread from each CWD core is likely to be independent, and the rates and patterns of disease spread may differ depending on the landscape features, deer movement, and contract rates around each infected area. Therefore, area-specific monitoring and management strategies may be necessary to limit the spread of disease from either the western or eastern core infection areas.

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