

Influence of genetic relatedness and spatial proximity on chronic wasting disease infection among female white-tailed deer

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Summary

1. Social organization and interactions among individuals are suspected to play important roles in the transmission and potential management of wildlife diseases. However, few studies have been conducted to evaluate sociality in wildlife disease transmission. We evaluated the hypothesis of socially facilitated transmission of chronic wasting disease (CWD) among adult female white-tailed deer using spatial location and genetic relatedness for 1387 female deer, and spatial locations of 1321 adult male deer harvested during 2002–2004 CWD control efforts in Wisconsin, USA.

2. Genetically related female deer were significantly clustered at distances of < 3.2 km. However, spatial autocorrelation based on maternally inherited mitochondrial DNA was 50-fold higher than relatedness estimated from microsatellite loci, indicating spatial overlap of females from different social groups with high rates of male-mediated dispersal and gene flow among groups.

3. Probability of CWD infection in adult females was significantly increased by closely related (full-sibling, mother-offspring) infected females that were both spatially proximate (≤ 3.2 km) and farther distant. To a minor extent, the probability of infection was also influenced by the number of nearby infected females (≤ 3.2 km), but not by the number of infected males.

4. Direct deer-to-deer transmission of CWD between closely related female deer may be an important route of local CWD transmission.

5. *Synthesis and applications.* Random mixing and infectious contact may be inadequate models for CWD transmission and disease spread in female deer. Frequency-dependent CWD transmission may be important for females because infectious contacts are limited between members of different female social groups, even if ranges overlap. Given that our data demonstrate a strong relationship between infection probability and female relatedness, CWD management should consider female harvest to maintain smaller female social groups and reduce contact among female deer. However, evaluation of the effects of this strategy on deer social behaviour and contact is needed.

Key-words: chronic wasting disease (CWD), disease ecology, epidemiology, microsatellite genetic markers, social structure, transmissible spongiform encephalopathy (TSE)

Introduction

Diseases are increasingly recognized as important processes affecting the ecology, population dynamics, life history and

conservation of many vertebrate species (Daszak, Cunningham & Hyatt 2000; Harvell *et al.* 2002). Traditionally, disease dynamics have been modelled assuming random mixing, where hosts have equal probability of contact and disease transmission (Anderson & May 1992). However, host characteristics such as size, familial relationships, composition of social groups, inter-group movement and inter-group isolation in space and time influence the likelihood and duration of

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contacts between hosts and contact with pathogens in the environment (Loehle 1995; Delahay *et al.* 2000). An important issue in disease ecology is how social organization influences infectious contacts and disease transmission (McCallum, Barlow & Hone 2001; Altizer *et al.* 2003; Cross *et al.* 2009). Although this information is critical for developing effective management actions (Keeling 1999; Cross *et al.* 2004), few wildlife studies have addressed the effects of sociality on disease transmission.

Chronic wasting disease (CWD) is an emerging neurological disease affecting North American deer (*Odocoileus* spp.), rocky mountain elk *Cervus elaphus* Baily and moose *Alces alces* Nelson, and CWD is the only known transmissible spongiform encephalopathy (TSE) that acts as an infectious disease in wildlife populations (Williams 2005). CWD has a lengthy incubation, followed by clinical signs and inevitable mortality; there is no known treatment (Williams 2005). Susceptibility to CWD infection and rate of disease progression appears to be affected by prion protein (PrP) genotype (O'Rourke *et al.* 1999; Johnson *et al.* 2003, 2006; Keane *et al.* 2008a). However, in our study area, >90% white-tailed deer *O. virginianus* Zimmerman have PrP genotypes known to be infected by CWD (Johnson *et al.* 2006). Studies of captive cervids have demonstrated that the CWD agent can be transmitted via animal-to-animal contact or by contact with a prion-contaminated environment (Sigurdson *et al.* 1999; Miller & Williams 2003; Miller *et al.* 2004). In captive mule deer *O. hemionus* Rafinesque, transmission via a contaminated environment appears to be more important than deer-to-deer contact (Miller, Hobbs & Tavener 2006). However, no studies have been conducted to determine the relative importance of deer-to-deer and environmental transmission in free-ranging deer. The routes and rates of CWD transmission in wild cervid populations are unclear (Gross & Miller 2001; Williams *et al.* 2002), making management of this disease difficult.

Social behaviours that influence contact rates in wildlife populations are potentially important factors in disease spread within social groups and among the entire host population (Altizer *et al.* 2003; Cross *et al.* 2009). White-tailed deer social structure is characterized by segregation of the sexes outside the breeding season and formation of matrilineal female groups outside the fawning period. Males have larger home ranges and more social interactions than females, increasing their chance of contact with infected deer or pathogen-contaminated environments (Hirth 1976; Miller & Conner 2005; Gear *et al.* 2006). Female interaction has been observed to centre on matrilineal groups comprised of an older female, her daughters and several generations of female offspring (Hawkins & Klimstra 1970). Successive generations of females establish home ranges that overlap natal ranges, have extensive range overlap, and interact socially with kin more frequently than with less-related individuals (Porter *et al.* 1991; Nelson & Mech 1999). Due to strong philopatry and lower dispersal of female white-tailed deer, matrilineal groups are expected to be genetically related and spatially clustered (Mathews & Porter 1993; Aycrigg & Porter 1997; Nelson & Mech 1999). Nonetheless, female social groups are not territorial and maintain varying degrees

of spatial overlap (Scribner *et al.* 1985; Aycrigg & Porter 1997; Comer *et al.* 2005; Schaubert, Storm & Nielson 2007). Higher male CWD prevalence in both adult mule deer (Miller & Conner 2005) and white-tailed deer (Gear *et al.* 2006) suggests that similar intraspecific social structure, but different social behaviour between the sexes, drives transmission dynamics. Further, male-biased infection patterns for *Mycobacterium bovis* (bovine tuberculosis) in Michigan white-tailed deer (O'Brien *et al.* 2002) and higher *M. bovis* infection among genetically related deer (Blanchong *et al.* 2007), imply that social behaviour may be important to understand infectious disease transmission in free-ranging deer populations.

Transmission is the most challenging epidemiological parameter to estimate for any host-pathogen system (McCallum *et al.* 2001). To compound this challenge, measuring infectious contact rates in most free-ranging animals is problematic, and traditional methods of measuring social structure (i.e. direct observation, radiotelemetry) limit the number of individuals and groups that can be studied. However, genetic methods allowed us to determine genetic relatedness among female deer removed from our study area and thereby evaluate the importance of social structure on CWD infection patterns (Scribner *et al.* 2005). Use of maternally inherited mitochondrial DNA and bi-parentally inherited microsatellite loci can provide valuable information regarding sex-specific gene flow (Scribner *et al.* 2001), and disease transmission. We hypothesized that CWD transmission was greater among related females in their matrilineal group. We focused on adult (≥ 1.5 years old) females because their matrilineal social organization suggests spatially structured genetic patterns and frequent contacts among related females, while high male dispersal rates predict little correlation between social interactions and genetic relatedness. We used multiple logistic regression models that combined genetic relatedness, spatial proximity and demographic information to evaluate CWD infection probability in individual adult female white-tailed deer in relation to age, genetic relatedness to infected females, spatial proximity of infected females and spatial proximity of infected males.

Materials and methods

STUDY AREA AND DATA COLLECTION

Joly *et al.* (2003) identified a 310-km² area in south-central Wisconsin, USA where CWD prevalence was higher (6.5%) than in surrounding areas (1.5%). White-tailed deer density in the area was estimated at 13.5–15.5 deer km⁻² (Rolley 2002) and deer have high fecundity, exhibit little within-season movement and females do not migrate seasonally (Larson, Rongstad & Tebilcox 1978; Ishmael 1984).

Deer were removed by hunter harvest and limited culling during April 2002–April 2003 and July 2003–January 2004. High harvest rates of male and female deer were common prior to our study and, therefore, we assumed our results represented genetic structure prior to, or were unaffected by, CWD management. The age, sex and kill location, assigned to the centre of a quarter section (65 ha, United States public lands survey system), were recorded for each harvested deer; giving a minimum spatial resolution of 0.8 km. Age was

determined by tooth replacement and wear (Severinghaus 1949). A portion of the brainstem (obex), retro pharyngeal lymph nodes (RPLN) and skeletal muscle were collected for CWD diagnosis and genetic analysis. RPLN and obex tissues were tested by immunohistochemistry (IHC) or plate ELISA (IDEXX Laboratories Inc, Westbrook, ME, USA) (Keane *et al.* 2008b). Positive ELISA tests were confirmed with IHC. Deer showing positive IHC results in the RPLN or the obex were classified as CWD positive.

CHARACTERIZATION OF MICROSATELLITE AND MITOCHONDRIAL GENOTYPES

A 5–10 g sample of muscle tissue from adult females was stored in 95% ethanol at -20°C pending DNA extraction using a QAIAGEN DNeasy spin-column procedure (Qiagen Genomics Inc, Bothell, WA, USA). Twelve polymorphic microsatellite loci: BM4107 and BM4208 (Talbot, Haigh & Plante 1996); Cervid1 and Cervid2 (DeWoody, Honeycutt & Skow 1995); RT-23, RT-7, RT-27 and RT-9 (Wilson *et al.* 1997); IGF1 (Kirkpatrick 1992); BM6506, BM1225, and CSN3 (Bishop *et al.* 1994) were chosen based on high-allelic diversity and absence of linkage. DNA was amplified using polymerase chain reaction (PCR) and electrophoresed on 6% polyacrylamide gels. Genotypes were visualized on a Hitachi FMBIOII gel scanner (MiraiBio Inc., Alameda, CA, USA) or LI-COR gel scanner (LI-COR Biosciences, Lincoln, NE, USA) and scored manually against known standards by two experienced laboratory personnel. We used exact tests with sequential Bonferroni correction in program GENEPOP (Raymond & Rousset 1995) to evaluate genotype frequencies for Hardy–Weinberg and linkage equilibrium.

We sequenced a 699 bp fragment of the mitochondrial DNA (mtDNA) control region (D-loop) on ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Primers were based on published sequences (Miyamoto, Kraus & Ryder 1990; accession no. ODOMTFVLA) including F1 (5'-TCT CCC TAA GAC TCA AGG AAG-3') and R1 (5'-GTC ATT AGT CCA TCG AGA TGT C-3'). PCR products were purified using Qiaquick (Qiagen Genomics Inc) and mtDNA sequences were generated using a nested primer F2 5'-ATC RCC CTG AAG AAR GAA CCA G-3'. Chromatograms were visualized and assembled automatically using BioEdit 7.0 (Hall 2004). All sequences were manually aligned and compared with a reference sequence and other sequences from the population to correct ambiguities and verify mutations. Descriptive statistics for haplotype and nucleotide frequencies, linkage equilibrium and other measures were calculated using Arlequin 2.00 (Schneider, Roessli & Excoffier 2000).

ESTIMATING PEDIGREE RELATIONSHIPS

We estimated pedigree relationships between female deer with likelihood ratio tests using program Kinship v 1.3.1 (Queller & Goodnight 1989). Queller and Goodnight's r_{xy} is an unbiased estimate of relatedness (range from -1 to 1) based on the population allele frequencies. A positive value indicates that a pair is more related, and a negative value indicates that a pair is less related than average. We identified closely related *kin* (full-sibling, mother-offspring) pairs of female deer based on primary and null hypotheses of full-sibling ($r_{xy} = 0.5$) vs. not related ($r_{xy} = 0$), respectively. All other pairs were considered *less-related*. Type 1 error rates (false positive) and type 2 error rates (false negative) were estimated via simulation of 350 000 pairs generated from observed allele frequencies (Goodnight & Queller 1999). Females identified as *kin* based on microsatellite genotype were excluded from this classification when they did not have matching

mtDNA haplotypes. We assumed female kin pairs were members of the same social group. We used a one-side Z-test with pooled variance estimated by jackknife procedures to test significance of the proportion of co-infected pairs within each category (*kin* and *less-related*) (Manly 2007). Jackknifed estimates of variance were used because the sample size of unique pairs was artificially large (961 191 pairs from 1387 individuals) and thus inappropriate for standard statistical tests.

ESTIMATING GENETIC RELATEDNESS AND SPATIAL DISTANCE

We examined spatial heterogeneity in genetic relatedness using the mean correlation of genetic distance based on the estimator from Lynch & Ritland (1999). This relatedness estimator has been shown to perform well under the conditions found in our study: many highly polymorphic loci and population substructure (Van De Castele, Galbursera & Matthysen 2001; Csillery *et al.* 2006; Oliehoek *et al.* 2006). We used the program GenALEx v6 (Peakall & Smouse 2005) to estimate relatedness among female deer within 0.8 km distance classes (our smallest spatial scale for deer locations). We estimated 95% confidence intervals (CI) around r for each 0.8 km distance class by bootstrap resampling ($n = 1000$) and a 95% confidence envelope for the hypothesis of no genetic structure in space by simulating a population of random pairwise linear distances based on observed genotypes ($n = 999$).

PROBABILITY OF INFECTION MODELS

We used multiple logistic regression models to evaluate whether factors related to exposure time (*age*), the number and proximity of infected males and females and the proximity of closely related infected females were associated with probability of CWD infection in adult female deer. We dichotomized spatial proximity between deer into ≤ 3.2 and > 3.2 km categories based on spatial autocorrelation of 3.6 km for CWD infection in our study area (Joly *et al.* 2006), correlation between genetic relatedness and distance (see Results), and the distance where regular contact or range overlap are unlikely based on home range size (Skuldt, Mathews & Oyer 2008). We used the number of infected-female kin (*kinNear*), infected less-related females (*nposFemale*) ≤ 3.2 km from each female, and the number of infected-female kin (*kinFar*) > 3.2 km from each female as independent variables. We tested logistic regression models to evaluate the importance of these independent variables in predicting the infection status of each adult female deer. A variance inflation factor (\hat{c}) was used to estimate overdispersion for our saturated model (Cox & Snell 1989) and we calculated global goodness-of-fit for the unweighted sum of squared errors (le Cessie & Van Houwelingen 1991) using 'lrm' in *R* (Harrell 2008). We used Akaike Information Criterion (AIC) to determine the relative importance and Akaike's weight of the competing models, and calculated model-averaged coefficients and predictions (Burnham & Anderson 2002). We assessed the effect of independent variables on the risk of CWD infection by calculating odds ratios (OR) and associated confidence intervals (Kahn 1983).

Results

Our analysis included 1387 adult females with disease status, location and microsatellite genotype of which 77 (5.5%) were CWD positive. Of these females, 1224 had mtDNA haplotype sequences, including 75 CWD positive females. There were

Table 1. Locus name, number of alleles, observed heterozygosity, expected heterozygosity and *P*-value for Hardy–Weinberg equilibrium (HWE) for 12 microsatellite loci genotyped in 1387 female white-tailed deer harvested in southern Wisconsin, April 2002–January 2004

Locus	No. of alleles	Heterozygosity		HWE <i>P</i> -value
		Observed	Expected	
BM1225	12	0.706	0.745	< 0.001 ^a
BM4107	16	0.810	0.810	0.978
BM4208	19	0.838	0.901	0.002 ^a
BM6506	13	0.703	0.871	< 0.001 ^a
IGF1	13	0.654	0.665	0.528
RT27	19	0.826	0.832	0.708
RT7	18	0.874	0.876	0.245
Cervid1	17	0.774	0.768	0.113
Cervid2	11	0.645	0.829	< 0.001 ^a
CSN3	6	0.328	0.477	< 0.001 ^a
RT23	19	0.913	0.912	0.694
RT9	10	0.816	0.800	0.009

^aLocus not in HWE after sequential Bonferroni correction.

1321 adult males with location and disease status, with 99 (7.5%) testing CWD positive. Observed male prevalence (7.4%) was significantly higher than female prevalence (5.4%) over all sampled deer in the study area (Grear *et al.* 2006). The

average age of our harvested females (excluding fawns) was 2.89 years old, with 80% of harvested females aged at 1.5–3.5 years old.

The number of alleles per locus ranged from 6 to 19 with 7 of the 12 microsatellite loci allele frequencies consistent with Hardy–Weinberg expectations (Table 1). We found 48 polymorphic mtDNA sites (nucleotide diversity = 0.0073), equating to 19 haplotypes (haplotype diversity = 0.3687). The mean number of pair-wise differences among these haplotypes was 4.217. Although most female deer (78%) shared a common haplotype, we found significant spatial structuring among less frequent and/or combinations of haplotypes (see below).

We identified 1287 kin pairs using microsatellite genotypes (type I error rate = 0.001, type II = 0.169) and excluded 313 (24%) of these pairs based on unmatched mtDNA haplotype sequences. Seven of 974 (0.72%) female kin pairs were co-infected compared with 2916 of 959 904 (0.30%) less-related pairs. We estimated the probability of co-infection to be 2.4 times higher (one-sided *Z* = 1.48, *P* = 0.07) in kin pairs than in less-related pairs.

GENETIC RELATEDNESS AND SPATIAL DISTANCE

We found higher microsatellite genotype relatedness than random among adult female deer harvested at ≤3.2 km, with relatedness declining from the smallest distance (Fig. 1a). Pair-wise

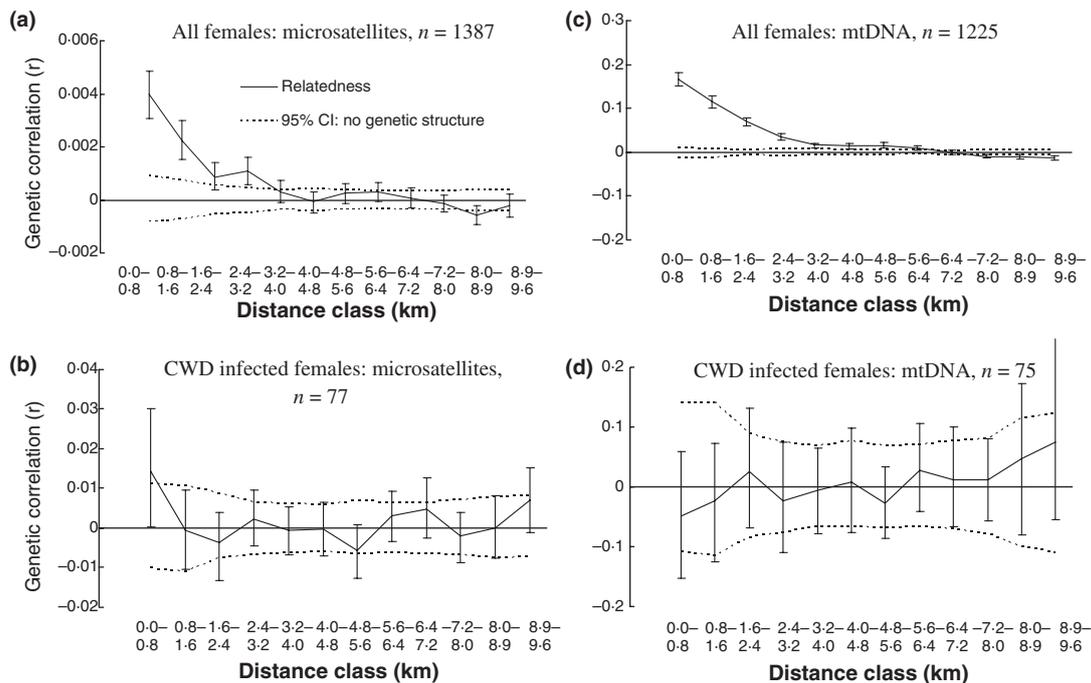


Fig. 1. Average genetic relatedness among adult female white-tailed deer harvested from core study area, April 2002–January 2004, in 0.8 km distance intervals based on 12 microsatellite markers (a and b) and mtDNA haplotype (c and d). Positive values indicate deer in the distance class are more related than the population average and negative values indicate individuals are less related than average. Error bars represent 95% confidence around genetic correlation (*r*-value) based on bootstrap resampling. (a) All harvested adult females with simulated 95% confidence bounds representing no genetic structure for simulated locations from observed microsatellite genotypes. (b) CWD positive individuals with simulated 95% bounds representing no genetic structure from observed microsatellite genotypes among CWD positive females. (c) All harvested adult females with simulated 95% confidence bounds representing no genetic structure from observed mtDNA haplotype. (d) CWD positive individuals with simulated 95% confidence bounds representing no genetic structure from observed mtDNA haplotype among CWD positive females.

Table 2. Top five alternative models for probability of CWD infection in adult female white-tailed deer ($n = 1387$) from southern Wisconsin, April 2002–January 2004

Model	AIC	Δ AIC	ω_i	Cummulative ω_i
$Age^a + kinNear^b + kinFar^c + nposFemale^d$	510.07	0.00	0.73	0.73
$Age + kinNear + kinFar + nposFemale + nposMale^e$	512.07 ^f	2.00	0.27	0.99
$Age + kinNear + kinFar + nposMale$	521.73	11.66	< 0.001	> 0.99
$KinNear + kinFar + nposFemale$	525.13	15.06	< 0.001	> 0.99
$Age + kinNear + kinFar$	526.77	16.70	< 0.001	> 0.99

ω_i refers to the probability that the model is the best candidate model given the data.

^aLinear relationship between age and prevalence.

^bNumber of infected female relatives within 3.2 km.

^cNumber of infected female relatives beyond 3.2 km.

^dNumber of infected females within 3.2 km.

^eNumber of infected males within 3.2 km.

^fFull model containing all independent variables.

mtDNA haplotypes were significantly higher than random among deer harvested at ≤ 3.2 km (Fig. 1c). Genetic correlation for mtDNA haplotypes was *c.* 50-fold greater than correlation based on microsatellite genotypes for distances < 3.2 km. CWD positive females had non-significant relationships between spatial distance and microsatellite genotypes or mtDNA haplotypes (Fig. 1b,d).

PROBABILITY OF INFECTION

Two logistic regression models were substantially better (Δ AIC ≤ 2) in predicting female infection than other models (Δ AIC ≤ 11) (Table 2). There was no evidence of overdispersion for the full model containing all independent variables ($\hat{c} = 1.04$, parameters = 5); therefore overdispersion corrections were not used for model selection or parameter variances. The two best models accounted for 99% of the Akaike weights and were used to estimate model-averaged parameters, variances and model predictions. The goodness-of-fit test indicated no evidence of lack-of-fit for the full model ($\chi^2 = 0.58$, d.f. = 1, $P = 0.56$). We found a significant positive relationship between the probability of infection and the number of nearby (≤ 3.2 km) infected-female kin ($kinNear = 4.93$, 95% model-averaged CI: 2.80, 7.10), the number of distant (> 3.2 km) infected-female kin ($kinFar = 3.30$, 95% model-averaged CI: 2.00, 4.50) and the number of nearby less-related-infected females ($nposFemale = 0.09$, 95% model-averaged CI: 0.04, 0.13). Female age ($age = 0.04$, 95% model-averaged CI: -0.10, 0.18) and the number of nearby infected males ($nposMale = -0.0002$, 95% model-averaged CI: -0.01, 0.01) appeared in the top models, but did not have significant model-averaged parameter estimates. Although, nearby highly related CWD positive deer ($kinNear$) were uncommon in our data set, the presence of one closely related CWD positive female < 3.2 km ($kinNear$) increased the probability of CWD infection (OR = 138.4, CI: 16.4–1212) to *c.* 80% compared with the background probability near 5% (Fig. 2). A CWD positive female > 3.2 km ($kinFar$) increased the probability of infection (OR = 27.1, CI: 7.4–90) to *c.* 50%. In contrast, increasing the number of CWD positive less-related females

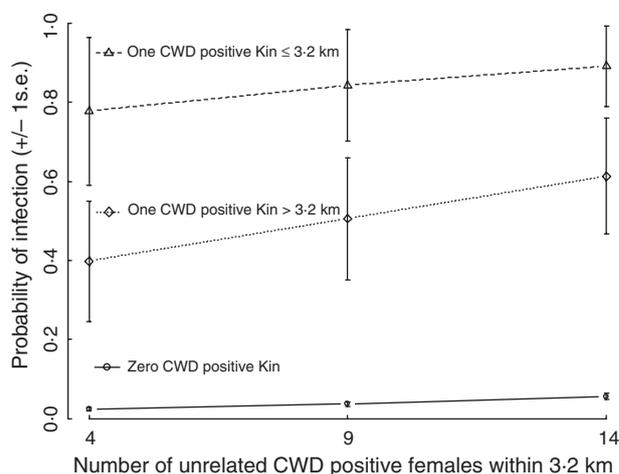


Fig. 2. Predicted probability of chronic wasting disease (CWD) infection for adult female white-tailed deer based on model-averaged parameters of top two candidate models (Table 2). Probability of infection for an average aged adult female deer (2.89 years) relative to the number of infected females within 3.2 km ($nposFemale$) with no CWD positive female relatives (circles), with one CWD positive female relative > 3.2 km ($kinFar$) (diamonds) and with one CWD positive female relative ≤ 3.2 km ($kinNear$) (triangles). Number of infected females based on 25th, 50th and 75th percentile of observed female infection covariate ($nposFemale$). Error bars represent one standard error from model-averaged predictions (Burnham & Anderson 2002).

< 3.2 km from 4 to 14 had relatively minor effects (OR = 2.5, CI: 1.5–3.7) on the predicted probability of infection (Fig. 2).

Discussion

GENETIC RELATEDNESS AND CWD INFECTION

Logistic regression models indicated that infection in female kin ≤ 3.2 km, infection in female kin > 3.2 km and the number of infected less-related females ≤ 3.2 km were significantly associated with CWD infection in adult female deer. The odds of CWD infection in adult female white-tailed deer increased by 138-fold when highly related deer, presumed to be in the same

matrilineal group, were also infected (Fig. 2). Such closely related kin have more contact, higher frequency and intensity of interaction, and greater spatial overlap compared with spatially proximate less-related females (Hirth 1976; Nelson & Mech 1999; Schaubert *et al.* 2007). Transmission among female kin > 3.2 km may have occurred during interactions as juveniles or during less frequent social contact throughout life. The weak association between CWD infection probability and the number of local infected females probably represents a measure of local disease risk. However, other routes of transmission that create this local risk in free-ranging deer populations, especially between females and males and via environmental sources, have not been adequately investigated (Grear *et al.* 2006).

We found significant decreasing relationships between measures of genetic relatedness and geographical distance among adult female deer harvested < 3.2 km apart. These relationships were much higher based on maternally inherited mtDNA haplotypes than bi-parentally inherited microsatellite genotypes; indicating considerable spatial overlap among female social groups and high levels of male-mediated gene flow (Scribner *et al.* 2001). Male-mediated gene flow across our 310 km² study area, encompassing many matrilineal groups, was likely to be responsible for observed deviance of Hardy–Weinberg expectations at 5 of 12 microsatellite loci (Table 1). There was also evidence for spatial genetic structure within this area (Fig. 2a,c; Blanchong *et al.* 2008). High overlap among female social groups is contradictory to clustered female social structure (Porter *et al.* 1991), and may result from higher harvest rates (Comer *et al.* 2005; Williams, DeNicola & Ortega 2008), high deer density or fragmented habitats (Blanchong *et al.* 2008) in our study region.

A small sample of CWD-infected relatives and overlap among social groups may have confounded our ability to detect a correlation between CWD infection and genetic similarity (Fig. 1b,d). However, the low degree of spatial genetic structure among CWD-infected deer may suggest a pattern of CWD prevalence distributed across many matrilineal groups. In this situation, infected deer would be scattered among less-related females, rather than clustered within few matrilineal groups. Alternatively, the low degree of spatial genetic structure among CWD-infected females could be a result of insufficient sampling of social groups. In this case, our comprehensive sampling may reflect adequate coverage across our study area, but comparatively few samples within social groups or for CWD-infected females, and may have diminished our ability to detect a higher occurrence of genetically related and co-infected females (Fig. 1). These issues may explain why we did not find spatial infection patterns consistent with highly structured social groups, like those observed for *M. bovis* infection in Eurasian badgers *Meles meles* (Delahay *et al.* 2000) or white-tailed deer in Michigan, USA (Blanchong *et al.* 2006, 2007).

The effects of white-tailed deer harvest on social structure, contact rates and the consequences for CWD transmission are not well understood. Williams *et al.* (2008) found that extreme density reduction of white-tailed deer (> 90%) may

cause females to join social groups of unrelated individuals, while less severe density reduction (65%) showed no evidence for social restructuring. Scribner *et al.* (1985) found no evidence for genetic restructuring under hunting methods similar to those in our study. Harvest rates of male and female deer in our study area have historically been moderate (30–50%) and designed to maintain a stable population density (Rolley 2002). There was no evidence that deer social behaviour (Skuldt *et al.* 2008) or CWD prevalence (Osnas *et al.* 2009) changed during our study. On the other hand, samples from these harvested deer allowed us to detect kinship associations within social groups, discriminate between spatial and kinship patterns with logistic regression, and provide evidence for a strong effect of social-group membership on infection probability.

INFECTION PATTERNS AND CWD TRANSMISSION

The strong influence of infected-female kin on the probability of CWD infection in conjunction with the small effect of infected less-related females, in close geographic proximity, suggests that direct transmission between related females during social interactions has greater impact on probabilities of CWD infection than a shared contaminated environment. Recent studies have demonstrated that saliva contains infectious prions, providing a potential route for direct transmission during social contact (Mathiason *et al.* 2006), and offspring of infected cow elk had an elevated risk of CWD infection on farms (Argue *et al.* 2007). The importance of more spatially distant female relatives on infection probability also supports the importance of social contact among females in the transmission of CWD because these animals probably have limited spatial overlap. Alternatively, these spatially distant female relatives could have become infected at a common natal area or by contact with infected kin prior to dispersal. Approximately 20–50% of female deer may disperse at 1 year of age (Nixon *et al.* 2007) with lower reported female dispersal in our study area (Skuldt *et al.* 2008).

We also found a positive, but weak relationship between probability of infection and the number of nearby infected less-related females. Unrelated and distantly related (aunts, grandmothers) females have lower rates of social contact compared with closely related (mother-daughter, sibling) individuals (Hawkins & Klimstra 1970; Nixon *et al.* 1991); therefore, it is plausible that associations between less-related females and probability of infection may represent environmental transmission or transmission from infrequent social interactions.

We acknowledge the challenges in detecting fine-scale genetic structure in our study population using 12 microsatellite loci and mtDNA sequences. Although estimation of pedigree relationships can usually be improved with more genetic information, Butler *et al.* (2004) found that eight loci with eight alleles each were sufficient for pedigree and full-sibling classification using algorithms similar to those we employed. To improve our kinship results and minimize type II error, we required that related kin had the same mtDNA haplotypes in addition to sufficient microsatellite genetic information to be considered kin. Despite some limitations, our results

demonstrate significant association between genealogy and CWD infection in female white-tailed deer. Considering the characteristics of CWD (long-incubation period, low prevalence, direct and indirect transmission), we do not believe standard longitudinal studies using telemetry or capture–recapture would be effective in addressing similar questions on disease transmission. We also note that our study may be one of the few to present both maternal and bi-parental genetic markers for a large-scale free-ranging wildlife population and we encourage other researchers to use similar methods to better understand social relationships and the breeding structure of natural populations.

Conclusions

In our study area, CWD has probably been present for more than two decades (Wasserberg *et al.* 2009) and is likely to have spread from a focal point of introduction (Joly *et al.* 2006). We found a high degree of concordance between female genetic relatedness and CWD infection at small spatial scales (3.2 km) and a weaker spatial correlation of CWD infection for all deer. Our results also indicate that CWD transmission among females is likely to be driven by social interactions among related individuals and less by spatial overlap with unrelated individuals. These patterns imply that CWD may behave more like a frequency-dependent disease because infectious contacts are socially constrained. Management of CWD based on deer reduction assumes that disease transmission is a density-dependent function of deer abundance (Schauber & Woolf 2003; Wasserberg *et al.* 2009). Our results suggest that CWD transmission among females is not strongly density-dependent and, therefore, generalized host reduction strategies may be ineffective in controlling CWD. As a result, CWD might not be eradicated without virtual elimination of deer (Gross & Miller 2001; Wasserberg *et al.* 2009) and, at higher prevalence, CWD may have substantial impacts on deer populations (Miller *et al.* 2008; Wasserberg *et al.* 2009). Factors that lead to large aggregations of deer (e.g. artificial feeding, winter deer yards, or seasonal concentration in fragmented habitats) may increase direct contact, as well as disease spread among matrilineal groups (Blanchong *et al.* 2006, 2007), and management actions that reduce such aggregations may be effective in slowing CWD spread.

Concordance of spatial patterns of CWD infection, spatial relatedness among female deer, and associations between genealogy and CWD infection probability suggest that a relationship exists between local disease transmission and female social structure that merits further investigation. Our results also suggest that CWD prevalence may increase more rapidly in deer populations that contain large matrilineal groups compared with many smaller groups because transmission would be more likely among many related individuals. Harvest of female deer is currently practiced in many states to maintain or reduce overabundant white-tailed deer populations and provides one management strategy to reduce the formation of larger matrilineal groups. However, we caution that increasing harvest rates on females for CWD management should include

research to determine the effects on female social behaviour. We also emphasize the need for research to understand the importance of the routes of transmission among wild cervids to improve our interpretation of epidemiology studies, our efforts to model CWD dynamics and our ability to formulate management actions.

Acknowledgements

This study could not have been completed without the time and hard work of the Wisconsin DNR staff and volunteers as well as the Wisconsin Veterinary Diagnostic Laboratory TSE testing staff. We thank C. Batha, T. Howard, M. Watrud, K. Beheler and J. Sausen of the WDNR and P. Boschler and D. Keane of the Wisconsin Veterinary Diagnostic Laboratory for the time and effort they put into sample collection, data management and disease testing. We thank K. Filcek, S. Libants, L. Main and A. Simon for their expertise and hard work in the Molecular Ecology Laboratory at Michigan State University. Funding was provided through the United States Geological Survey. We also thank D. Colman, M. Conner and E. Schaubert for valuable comments on early drafts of this manuscript.

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Received 2 September 2009; accepted 11 March 2010

Editor: E. J. Milner-Gulland