Utilizing disease surveillance to examine gene flow and dispersal in white-tailed deer

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Summary

1. The prevention and management of transmissible diseases hinges upon understanding host dispersal because it influences distribution of wildlife, affects the rate of disease transmission, and alters the spatial distribution of infection. The relationship between host dispersal and chronic wasting disease (CWD) in cervids is of interest because potential interspecies transmission of fatal prion diseases creates serious risks for wildlife, domestic species, and humans.

2. We used molecular techniques to examine dispersal in a population of Illinois white-tailed deer Odocoileus virginianus. Sampled individuals inhabited areas with confirmed cases of CWD, a transmissible prion disease of cervids, with additional sampling in uninfected locations. We genotyped 1410 deer harvested through CWD surveillance using 10 microsatellites and measured gene flow, determined population structure and quantified gender-specific differences in dispersal. Additionally we used spatial autocorrelation and parentage assignments to examine individual movements.  

3. Female deer demonstrated philopatry as evidenced by higher levels of genetic structure, positive spatial autocorrelation and maternity assignments within one home range. 

4. Male deer were less genetically structured and frequently exchanged genes across >100 km. 

5. Synthesis and applications. Dispersal contributes to the spread of wildlife diseases. Therefore, knowledge of wildlife movement patterns can enhance the efficacy of disease control programmes. Our findings show that samples collected for disease surveillance are useful for measuring gene flow and inferring dispersal in white-tailed deer. High genetic admixture indicates males disperse regardless of landscape features. In contrast, distinct clustering of females demonstrates localized dispersal and philopatry. Taken together, results suggest that CWD surveillance and culling of males should be broadly expanded after an outbreak. Furthermore, surveillance of hunter-harvested deer can be used to identify locales in which CWD occurs, and this information should be used to focus culling efforts on females within genetically defined clusters (‘matriarchal groups’). Removal of matriarchal groups at those locations will reduce horizontal transmission more than widely distributed population reductions.

Key-words: genetic structure, isolation by distance, microsatellite, prion, sex-biased dispersal, spatial autocorrelation

Introduction

Dispersal has long been considered the foundation of ecology (Andrewartha & Birch 1954) and is equally important to epidemiology as it determines interconnectivity among animals, humans, and zoonotic pathogens. As wildlife hosts and vectors move through the landscape they influence the rate of disease spread, spatial extent of infection and likelihood of new outbreaks (Cullingham et al. 2008). Because it impacts spatial and temporal disease dynamics, dispersal must be carefully considered when implementing disease control strategies as it
will influence the effectiveness of management programmes. Interconnections between disease and dispersal can make wildlife management difficult particularly when interspecies contacts are frequent in landscapes where urbanization, agriculture, and wildlife habitats overlap (Bennett, Radford & Haslem 2006).

An understanding of dispersal in white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780) is critical for the management of chronic wasting disease (CWD), a prion disease of cervids. Chronic wasting disease in North America has caused economic losses associated with reduced hunting, depopulation of farmed cervids and loss of international markets (Arnot et al. 2009). Outbreaks of CWD in the U.S. have occurred near cities and agricultural farms which can create opportunities for zoonotic prion disease transmission (Macdonald & Laurensen 2006). While CWD associated prion disease has not been documented in humans, transmission of bovine spongiform encephalopathy (BSE) as variant Creutzfeldt–Jakob disease (vCJD) and experimental infection of cattle with CWD suggest that species barriers for prion diseases are limited (Belay et al. 2004). Chronic wasting disease management is therefore essential to protect wildlife resources and minimize risks to humans and livestock. As a result several states have implemented disease surveillance and management plans in captive and wild cervids. Wildlife managers need information on deer movement to determine the risk of prion spread among habitats and into urban and agricultural landscapes in order to implement the most effective strategies for disease control.

Radiotelemetry studies have suggested that dispersal is male-biased with fawns and females demonstrating philopatry (Marchinton & Hirth 1984). Dispersal distances for radiocollared deer in Illinois ranged from 28–44 km and most deer did not disperse > 50 km (Nixon et al. 2007). Unfortunately, telemetry studies are labour intense and limited by sample size, study area, and the difficulties of trapping a random sample (Koenig, Van Vuren & Hooge 1996). Recently, genetic measures of dispersal have gained popularity because population-level movements can be examined across entire landscapes to provide essential information for ecosystem-based management plans. Habitat connectivity can be assessed by quantifying gene flow among populations (Berry, Tocher & Sarre 2004). Furthermore, Bayesian assignment tests (Pritchard, Stephens & Donnelly 2000) and parentage analyses (Kalinowski, Taper & Marshall 2007) can assign individuals to demes, and quantify geographic distances between parent-offspring pairs.

We applied genetic methods to assess movements of white-tailed deer, a species that has been intensely managed and hunted for decades (Calhoun & Loomis 1974). In this study we utilized tissues from deer harvested through CWD surveillance and management programmes aimed at reducing further CWD transmission. Our goals were to:

1. Examine population-level movements and test for male-biased dispersal by quantifying gene flow among populations.
2. Identify genetic clusters and determine admixture with individual-based Bayesian assignment tests.
3. Examine dispersal using spatial autocorrelation and parentage assignment.
4. Evaluate the feasibility of indirectly measuring dispersal using samples collected through wildlife management programmes.

Our study provides a rare opportunity to assess indirect measures of movement in the context of wildlife disease management. Our genetic evaluation of behaviour also allows comparison to previous studies of deer dispersal employing direct measures, thus broadening our understanding of deer ecology and contributing to adaptive management of wildlife diseases.

**Materials and methods**

**DEER SAMPLING**

We utilized tissues collected through CWD surveillance and population control programmes targeting four areas in Illinois: north-western, north-central, north-eastern, and east-central. The aim of the surveillance programme is to improve confidence in disease detection and evaluate management strategies (Thurmond 2003). The majority of samples were from areas at increased risk of disease based on proximity to prior CWD cases. Additional samples were from areas of special management interest. The data comprised harvest date, gender, age, and spatial locations.

**North-central Illinois sampling area (NIL)**

Between January 2003 and March 2008, Illinois Department of Natural Resources (IDNR) harvested c. 5000 free-ranging deer in the CWD-infected region of northern Illinois (Fig. 1 and inset). Of the samples collected, 814 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. For deer sampled in NIL, spatial locations were recorded to the nearest township/range/section (TRS). For population-level analyses the NIL sampling area was subdivided into 13 similarly-populated study sites based on geographic distribution of sampled animals (Fig. 1 and inset).

**North-western Illinois sampling area (GTA)**

Between January and February 2008, 222 free-ranging deer were harvested in population control programmes from Galena Territory Association in Jo Daviess County. For deer sampled in GTA, spatial locations were recorded to the nearest 0:1 km.

**North-eastern Illinois sampling area (DuP)**

Between December 2007 and January 2008, 50 free-ranging deer were harvested by United States Department of Agriculture Wildlife Services personnel through deer management programmes initiated by the DuPage County Forest Preserve. For deer sampled in DuP, spatial locations were recorded to the nearest township/range/section (TRS).

**East-central Illinois sampling area (RAP)**

During the autumn hunting seasons between 2005 and 2007, 324 free-ranging deer were harvested at University of Illinois Robert Allerton Park (RAP) through their Deer Management and Research
Programme. For deer sampled in RAP, spatial locations were recorded to the nearest kilometre.

LABORATORY PROCEDURES

Genomic DNA was extracted from ethanol-preserved muscle samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Individuals were genotyped using microsatellite primers developed for white-tailed deer (Anderson et al. 2002; Blanchong 2003). This panel included markers BM1225, BM4107, CSN3, (Bishop et al. 1994), IGF-1 (Kirkpatrick 1992), OBCAM (Fries, Eggen & Womack 1993), OarFcb304 (Buchanan et al. 1993), RT20, RT23, RT27 (Wilson et al. 1997), and Srcrsp-10 (Bhebhe et al. 1994).

Mutations in microsatellite flanking regions required the re-design of primers CSN3 (reverse primer = TAGCTCATAATGTAAACCACTTT) and RT20 (forward primer = TGGAAGATTTCAGAAATGAT). Forward primers were labelled with fluorescent dyes (NED, HEX, FAM) with fragments separated on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA, USA) and visualized with genemapper (v. 4.0; Applied Biosystems). We used micro-checker (v. 2.2.3; Van Oosterhout et al. 2004) to evaluate genotyping errors using expected allele frequencies derived under Hardy–Weinberg equilibrium (HWE).

DESCRIPTIVE STATISTICS

Expected heterozygosity was calculated for each locus in each of the four sampling areas (with all 13 NIL study sites pooled) using arlequin version 3.1 (Excoffier, Laval & Schneider 2005). Allelic diversity and the number of rare alleles per locus (alleles exclusive to one sampling area) were determined with deviations from HWE examined using $F_{IS}$ (inbreeding coefficient) by locus and overall with genepop (v. 4.0; Rousset 2008).

POPULATION-LEVEL MOVEMENTS

We computed $F_{ST}$ values among study sites with Arlequin (Excoffier, Laval & Schneider 2005) and used Mantel tests to examine correlations with geographic distance (in km) using Isolation by Distance Web Service (IBDWS v. 3.16; Jensen, Bohonak & Kelley 2005), with significance based on 1000 random permutations. Isolation by distance (IBD) was examined at two spatial scales: distances < 100 km including all 13 NIL study sites and distances < 300 km encompassing NIL, DuP, GTA, and RAP study sites. When performing tests for IBD, we analysed both genders combined, then each separately.

We tested for sex-biased dispersal using FSTAT (v. 2.9.3.2; Goudet 2001) with $F_{ST}$ values calculated separately for males and females at < 100 km and < 300 km spatial scales and significance determined by 1000 random data-permutations. To further define gender-specific population boundaries, our 16 study sites (Fig. 1) were coalesced into populations based on homogeneity of allele frequencies determined by TEGPA (v. 1.3; Miller 1997). To approximate probabilities associated with observed allele frequencies for each locus, chi-squared tests were calculated from contingency tables of allele frequencies among all pairwise combinations of study sites and significance was evaluated using Markov Chain Monte Carlo (MCMC). For each single-locus test, 5000 permutations were completed for each of 20 batches with 1000 dememorization steps. $P$-values from single-locus tests were pooled using Fisher’s combined probability test and multi-locus $P$-values (< 0.05) were evaluated for departures from homogeneity in allele frequencies between populations. To prevent multi-locus tests from being dominated by a single marker, single-locus $P$-values were minimized at $P = 0.0001$ (Waples & Gaggiotti 2006).

Study sites not significantly different from one another in the contingency tests were then combined as samples representing the same genetic population. Thus, populations may consist of one to several study sites for which all other multi-locus tests were significant, or
instead as multiple study sites linked through a series of non-significant tests (Waples & Gaggiotti 2006).

**BAYESIAN ASSIGNMENT OF INDIVIDUALS**

We also inferred population structure using an individual-based Bayesian clustering method implemented in STRUCTURE (v. 2.3.1; Pritchard, Stephens & Donnelly 2000) that estimates the natural logarithm of the probability [\(\ln P(D)\)] that individual genotypes belong to a given cluster (\(k\)), thus negating the need for a priori population definitions. Twenty replicates were run for \(k\) values 1 through 7 and the simulation yielding the smallest Bayesian deviance was selected as the optimal cluster model (Pritchard, Stephens & Donnelly 2000; Falush, Waples & Gaggiotti 2007). An admixture model (initial \(\alpha = 1.0\), max \(\alpha = 100\), SD of \(\alpha = 0.05\)) was computed with TRS as the location for each deer and correlated allele frequencies specified to account for shared ancestry (Pritchard, Stephens & Donnelly 2000; Falush, Stephens & Pritchard 2003). Initially, several pilot simulations were run for each data set to determine burn-in length and consistency of parameter estimates across replicates. Male and female clusters were then simulated separately using 100 000 MCMC burn-in steps followed by 100 000 replicates to estimate posterior probabilities of all parameters. Individuals were assigned to the inferred cluster containing the highest percentage of membership (\(q\)).

**SPATIAL AUTOCORRELATION**

Tests for global spatial autocorrelation were performed with individuals from NIL and DuP using Spatial Genetic Software (v. 1.0d; Degen, Petit & Kremer 2001). Those from GTA and RAP were omitted due to inadequate sampling at intermediate distances. Individuals were grouped by gender and age (fawn, yearling or adult) so that sex-biased dispersal and juvenile movements could be examined. For all individuals, pairwise geographic distances were calculated from the \(x/y\)-coordinates of the TRS centroid (2.6 km\(^2\)) from which they were sampled. Moran’s I was calculated for each group at distances ranging from 2–200 km. To evaluate significance, a one-tailed distribution was derived from 1000 random permutations of the data. Distance classes were 0–2 km (between adjacent TRS), 0–3 km (separated by 1 TRS), 0–6 km, 0–12 km, 0–24 km (c. 1 dispersal event; Nixon et al. 2007), 0–48 km, 0–100 km (long-distance dispersal). Sample sizes for each group at each of the 8 distance classes are in Table 1.

**PARENTAGE ANALYSIS**

Mother-offspring pairs were identified using CERVUS 3.0 (Kalinowski, Taper & Marshall 2007). CERVUS employs a multi-step process to first simulate the distribution of log-likelihood values for mother-offspring pairs using allele frequency data from sampled populations, and secondly to determine statistical confidence of parentage for sampled individuals. To ensure conservative parentage estimates, an error rate of 0.001 was used (observed error rate = 0.005, data not shown) in simulations of 100 000 offspring. All females were used as candidate mothers while all individuals were considered potential offspring with subsequent removal of duplicate parentage assignments for a single pair of individuals. We validated the ability of our markers to resolve parentage by including 100 confirmed mother-offspring pairs (mothers with foetuses in utero). Based on simulations of the observed data and validation with mother-offspring pairs, assignments at 90% confidence were evaluated. Spatial distances among mother-offspring pairs were determined using the geographic distance calculation in GENALEX (Peakall & Smouse 2006).

**Results**

**DESCRIPTIVE MEASURES**

Multi-locus genotypes were obtained for 1410 deer across four sampling areas in the northern half of Illinois. None of the 10 microsatellites violated HWE assumptions. Only one marker in NIL showed significant heterozygote deficiency, but \(F_{IS}\) was low (\(F_{IS} = 0.019\)) suggesting subpopulation structure rather than inbreeding. Global tests showed no evidence of heterozygote excess in any of the sampling areas. Number of alleles per locus (average = 12) ranged from 2 (OarFcb304) to 19 (RT23), with 15 rare alleles observed across 6 loci in NIL and RAP (Table 2).

**POPULATION-LEVEL MOVEMENTS**

IBD was detected for all individuals at 100 km (Mantel \(r = 0.39, P = 0.03\)), but not at 300 km (Mantel \(r = 0.27, P = 0.11\)). When genders were analysed separately, females demonstrated IBD at the 100-km spatial scale (Mantel \(r = 0.37, P = 0.04\)), but not at 300 km (Mantel \(r = 0.15, P = 0.24\)). Males did not exhibit IBD at either spatial scale (Mantel \(r = -0.03, P = 0.54\) and 0.15, respectively).

Average female \(F_{ST}\) at 100 km (\(F_{ST} = 0.0122\)) was larger than that of males (\(F_{ST} = 0.0054; P = 0.004\), underscoring male-biased dispersal (Marchinton & Hirth 1984). Similar patterns were observed at 300 km, with females genetically more structured than males, again with a larger \(F_{ST}\) (\(P = 0.009\); Fig. 2).

### Table 1. Sample sizes (\(n\)) and number of pairwise comparisons at each distance class for global spatial autocorrelation of deer grouped by gender and maturity (\(M = \text{male}, F = \text{female}\)) in northern Illinois (NIL) and DuPage County (DuP)

<table>
<thead>
<tr>
<th>Group</th>
<th>Distance class</th>
<th>0–0.5</th>
<th>0–2</th>
<th>0–3</th>
<th>0–6</th>
<th>0–12</th>
<th>0–24</th>
<th>0–48</th>
<th>0–100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult M</td>
<td></td>
<td>90</td>
<td>96</td>
<td>135</td>
<td>146</td>
<td>286</td>
<td>582</td>
<td>1271</td>
<td>2402</td>
</tr>
<tr>
<td>Yearling M</td>
<td></td>
<td>85</td>
<td>125</td>
<td>227</td>
<td>297</td>
<td>449</td>
<td>597</td>
<td>1179</td>
<td>2027</td>
</tr>
<tr>
<td>Fawn M</td>
<td></td>
<td>164</td>
<td>340</td>
<td>590</td>
<td>772</td>
<td>1845</td>
<td>2650</td>
<td>5593</td>
<td>10226</td>
</tr>
<tr>
<td>Adult F</td>
<td></td>
<td>299</td>
<td>1063</td>
<td>1843</td>
<td>2267</td>
<td>4671</td>
<td>7420</td>
<td>15152</td>
<td>29495</td>
</tr>
<tr>
<td>Yearling F</td>
<td></td>
<td>69</td>
<td>126</td>
<td>174</td>
<td>189</td>
<td>270</td>
<td>402</td>
<td>726</td>
<td>1260</td>
</tr>
<tr>
<td>Fawn F</td>
<td></td>
<td>179</td>
<td>753</td>
<td>1067</td>
<td>1259</td>
<td>2264</td>
<td>3029</td>
<td>5003</td>
<td>8495</td>
</tr>
</tbody>
</table>
Males revealed weak genetic structure based on contingency tests for heterogeneity of allele frequencies among study sites. Two (of 16; 12.5%) study sites (RAP and GTA) were significantly distinct populations \((P < 0.05)\). NIL and DuP study areas were linked through a series of non-significant tests, thus indicating a single panmictic population (Fig. 3). This population encompassed a metropolitan area with an extensive network of interstates and multi-lane highways, predominantly running north to south. For males, allele frequencies were the same across major interstates, and on all sides of Rockford, IL, indicating that these landscape features did not act as barriers to male movement.

Females were significantly more structured than males across the 16 study sites, with seven genetically heterogeneous populations (43.75%; Fig. 3). Allele frequencies in RAP, GTA, and four NIL study sites were heterogeneous \((P < 0.05)\) when compared to all other study sites. The remaining nine NIL and DuP study sites were compiled into one genetic population through a series of non-significant tests.

### Table 2. Expected heterozygosity, sample size \((n)\) and the number of alleles (both common and rare to one sampling area) detected in Illinois deer

<table>
<thead>
<tr>
<th>Study site</th>
<th>n Females</th>
<th>n Males</th>
<th>Expected heterozygosity*</th>
<th>Number of alleles*</th>
<th>Number of rare alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIL</td>
<td>485</td>
<td>320</td>
<td>0.72</td>
<td>117</td>
<td>8</td>
</tr>
<tr>
<td>GTA</td>
<td>150</td>
<td>60</td>
<td>0.7</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>RAP</td>
<td>226</td>
<td>76</td>
<td>0.72</td>
<td>113</td>
<td>6</td>
</tr>
<tr>
<td>DuP</td>
<td>19</td>
<td>17</td>
<td>0.73</td>
<td>87</td>
<td>0</td>
</tr>
</tbody>
</table>

*Average across 10 loci.

NIL, northern Illinois; GTA, Galena Territory Association; RAP, Robert Allerton Park; DuP, DuPage Country Preserve.

For females, allele frequencies were heterogeneous for study sites separated by north-south interstates of metropolitan Rockford and for adjacent study sites (study site 10 and 11) separated by interstate I-39, implicating these landscape features as barriers to female movement.

### Bayesian Assignment of Individuals

Consistent with results from contingency tests, for males a 3-cluster model produced the lowest deviance value in STRUCTURE (Fig. 4). The first cluster included RAP, a second GTA, and a third DuPi + all 13 NIL study sites (inferred clusters denoted by italics and subscript ‘i’). Figure 3 shows the spatial distribution of males assigned to each of the three clusters, with misassignment rates ranging from 0 to 31% (Fig. 3). The NIL/DuPi cluster included males from opposite sides of Rockford and interstates, further emphasizing that these landscape features did not impede male movement (Fig. 3a, inset).

Males from RAP were the most genetically distinct (90% membership therein), whereas GTA was admixed with 66% assigned therein, 18% to RAP, and 16% to the NIL/DuPi. Males from NIL and DuP had intermediate levels of admixture with 71–51% membership assigned to NIL/DuP. Overall, southern study sites were more admixed than northern sites in NIL/DuP. Study sites assigned to NIL/DuP were of mixed ancestry with RAP, in that 12 (of 14) study sites reflected RAP, rather than GTA, membership. Across all replicates at \(k = 3\) clusters, male assignments were consistent, membership estimates stable, and all inferred clusters were in HWE for all loci \((P < 0.01)\).

For females, four clusters produced the lowest Bayesian deviance value (Fig. 4). Although all four were in HWE \((P < 0.01)\), study site assignments varied within replicates, and membership proportions were inconsistent. Consistent with population boundaries determined through contingency tests, the best fit model placed RAP into one cluster and GTA into a second cluster. The third cluster contrasted contingency tests by amalgamating DuP, nine NIL study sites north and east of Rockford, and one study site (11) to the south of Rockford into one cluster. The last inferred cluster \((NILSW)\) also contrasted contingency tests by amalgamating three genetically heterogeneous populations to the south and west of Rockford (study sites 5, 12, 13) into one cluster. Figure 3 shows the spatial distribution of individuals assigned to the four inferred clusters. Misassignment rates for females ranged from 0 to 47%. Females from the RAP study area were the most genetically distinct (91% membership), whereas females from GTA were also highly differentiated (86% membership) with the remaining 14% assigned preferentially to the NIL/DuP cluster. For study sites in NIL/DuP, admixture became elevated as study sites approached Rockford, a pattern consistent with IBD and clinal admixture in this area.

### Spatial Autocorrelation

Adult males were not spatially structured at distances ≤100 km, though male yearlings were structured at 0–6 and...
For male fawns, significant positive autocorrelation was observed at 0–2 km. Adult females also showed positive spatial autocorrelation that was maintained at distances £48 km, with gradual decreases in Moran’s I observed with increasing distance (Fig. 5). While female yearlings were not spatially autocorrelated at any distance, female fawns were significantly autocorrelated at 0–2, 0–12, and 0–24 km distance classes.

**PARENTAGE ANALYSIS**

Only 4% of non-mothers were assigned maternity at 90% confidence when the true mother was not sampled, and 6% when the true mother was sampled. All mother-foetus pairs used for validating parentage were correctly assigned at 90% confidence levels. For individuals with unknown parentage, cervus assigned 10 mother-offspring pairs at 90% with no mismatches across all multi-locus genotypes. Of the 10 pairs assigned, five were mother-daughter pairs and five were mother-son pairs. All assigned male offspring were £2 years old, with 80% of mother-son pairs being fawns. Two mother-daughter pairs included fawns, while the remaining three assignments were between adult females. Distances between assigned pairs ranged from 1 to 4 km with an average distance of 2.9 km. Half of the mother-offspring pairs detected were within the same home range, while the other half were separated by distances equivalent to adjacent home ranges (per Nixon et al. 1991 for deer in RAP).
Predicting the spread of wildlife disease and identifying areas at high risk for infection hinges upon quantification of animal dispersal (Castillo-Chavez & Yakubu 2001). Many ecological and epidemiological studies have focused on cervids because their elevated mobility can rapidly spread disease to new locations (Conner et al. 2008; Blanchong et al. 2008). Furthermore, cervids frequently cohabit with livestock and also reside in urban areas, thus providing opportunities for zoonotic disease transmission (Dazak, Cunningham & Hyatt 2000). Utilizing genetic samples obtained through disease surveillance programmes, we quantified deer movements in areas infected with CWD. Admixture proportions calculated from assignment tests suggest that long-distance dispersal (≤300 km) occurs and that CWD could spread across the landscape through occasional long-distance movements. These findings implicate long-distance dispersers as potential carriers of disease, and are consistent with the current distribution of CWD in Illinois where isolated cases are detected > 100 km from the outbreak focus near Rockford (Illinois Department of Natural Resources 2008; Fig. 1).

Our genetic analyses suggest that males have potential to spread disease because of their extensive local dispersal (<100 km). More importantly, the genetic structure of males suggests that a substantial proportion of the population disperses >100 km, which in turn, implicates them as vehicles for long-distance transmission. Dispersing males often form bachelor groups, and infectious prions could be transmitted through saliva during male grooming behaviours (Marchinton & Hirth 1984; Mathiason et al. 2006). Alternatively, sick animals can shed prions into the environment and contaminate the areas they traverse (Williams & Miller 2002). During the rut, male deer often scrape the ground and then urinate, or rub branches with their antlers to mark territories (Marchinton & Hirth 1984). Urine (Gonzalez-Romero et al. 2008) and antler velvet (Angers et al. 2009) can contain infectious prions. These behaviors could be facilitating environmental transmission of CWD because multiple deer visit rubs and scrapes within a season (Marchinton & Hirth 1984).

In contrast to males, spatial autocorrelation and parentage assignments indicate that females are philopatric, suggesting that they are more likely to spread diseases locally within their cohort. Thus Grear et al. (2010) reached a similar conclusion for female deer in Wisconsin. Although, these authors were unable to observe spatial autocorrelation at more than 32 km, possibly because of Hardy–Weinburg disequilibrium or low sampling density. Nonetheless, similar outcomes from geographically different areas strengthen this conclusion. Social contact among female relatives in close proximity is associated with elevated risk of CWD, because of increased horizontal transmission or increased environmental exposure to CWD within shared natal ranges (Grear et al. 2010).

Sites near agricultural habitats (such as those to the northeast of the city) were generally highly admixed for both male and female deer and corresponded to the areas of initial CWD outbreak and early rapid spread of disease. Furthermore, admixture between deer from northern Illinois and GTA was higher than admixture between northern Illinois and GTA. Although the distance to GTA is greater, the intervening landscape to GTA which is more intensively agricultural, in contrast to the intervening landscape to GTA which is more heavily forested. Genetic exchange is elevated to the south consistent with the tendency for southerly spread of CWD. Though preliminary, disease spread appears to parallel gene flow, and our results support the concept of CWD transmission following dispersal of infected animals.

Uninfected deer populations experiencing high genetic admixture with infected locations represent areas at high risk for future infection unless dispersal is unidirectional away from uninfected locations. Such locations, for example DuP in this study, are of particular interest for further research, both to monitor for future infection and to understand processes that have enabled them to remain uninfected despite high admixture with infected areas. Our data show that genetic structure in deer is shaped by differences in gender-specific dispersal with male-biased dispersal evident in $F_{ST}$ values and spatial autocorrelation patterns. Overall males were genetically homogeneous locally (<100 km) and slightly admixed regionally (<300 km), which in turn demonstrates their enhanced dispersal capabilities. In contrast, females were genetically structured locally (<48 km) according to spatial autocorrelation and contingency tests, indicating reduced connectivity among philopatric subpopulations.
Collectively, female philopatry is responsible for genetic structuring at distances <100 km whereas male dispersal is primarily responsible for connectivity among habitats separated by ≤300 km.

Our results also indicate that males and females respond differently to habitat fragmentation induced by urbanization, suggesting disease spread into urbanized areas may be gender-based. We detected barriers for female dispersal between geographically proximate study sites using FST and contingency tests. Female structure corresponded to habitat isolation induced by an interstate freeway (I-39: a 4 lane, divided and fence roadway with limited vehicle access) built in the 1980s. However, males reflected homogeneity of allele frequencies at these same study sites (Fig. 3) suggesting the freeway does not limit connectivity for males at this location. Furthermore, the panmictic population quantified in northern Illinois implies that males disperse freely within this 6900 km² area, apparently undeterred by Rockford, the third largest city in Illinois. Females, on the other hand, were inhibited by Rockford, as female gene flow was reduced and separate populations were detected on each side of this urbanized area.

Our results for females contradict those from a deer study in Wisconsin (Blanchong et al. 2008) where female dispersal was not deterred by roads. However, the road was a highway (US18/151: a divided 4-lane road, but with high vehicle access) which presents less of a physical barrier than an interstate freeway. Our findings are consistent with Epps et al. (2005) who showed that gene flow in big horn sheep Ovis canadensis was truncated by interstate highways while Pérez-Espona et al. (2008) found that red deer movement was deterred by high traffic roads. These anthropogenic alterations of wildlife habitats promoted genetic structuring over a period of 20–40 years (Epps et al. 2005).

Dispersal, as quantified in our study through gene flow, did not always agree with direct estimates of dispersal for Illinois deer. We did not detect positive autocorrelation in female yearlings, suggesting that they had dispersed at the time of sampling. This is in contrast to other studies of female deer reporting philopatry (Hawkins & Klimstra 1970). However, in our study only 69 yearling females were sampled, and this number is low compared to other studies (Hawkins & Klimstra 1970; N = 79). Additionally, STRUCTURE results showed that ongoing genetic exchange occurs at distances >200 km (Fig. 3), even though long-distance dispersals (c. 200 km) are rarely documented in ecological studies of deer (Brinkman et al. 2005; Oyer, Matthews & Skuld 2007). Further, while male-biased dispersal was detected with our genetic data, telemetry data suggests that both sexes disperse in Illinois (Nixon et al. 1991; Hansen, Nixon & Beringer 1997; Nixon et al. 2007). In agricultural habitats of Midwestern North America similar proportions of radiocollared deer dispersed from their natal ranges in RAP (57% of males and 49% of females), and in DeKalb County (68% of males and 45% of females) (Nixon et al. 2007). Goudet, Perrin & Waser (2002) argued that sex-biased dispersal must be stronger than that documented (for example) by Nixon et al. (2007) before it can be detected using genetic data. Nevertheless, our study was performed at the population-level and included >1400 deer sampled across the northern half of Illinois. Given this, we were able to detect long-distance dispersals and subpopulation processes that are often overlooked in short-term ecological investigations.

Discretion must be used when gene flow estimates are employed as a surrogate for dispersal in mobile organisms, especially with samples obtained from disease surveillance. In Illinois, surveillance is concentrated in areas with CWD, therefore, our geographic distribution of samples is limited. Given the high genetic admixture observed, the spatial scale examined may not capture the potential for gene flow across the region. Sampling at a larger spatial scale or across a wider array of habitats would have allowed us to more accurately quantify long-distance dispersals and landscape barriers to deer movement across the Midwest. Also, because we sampled to detect disease, rather than continuously across the landscape, uneven sampling may have affected the likelihood of detecting mother-offspring pairs and spatial autocorrelation. Hence, while parentage analysis allowed us to identify mother-offspring pairs within a single home range, our sampling distribution probably prevented identification of pairs at larger distances. Nevertheless, we applied conservative criteria to detect parentage in that simulated error rates were five times lower than observed. In addition, when performing spatial autocorrelation and parentage, we carefully subsampled the data and omitted deer from GTA and RAP so as to prevent bias due to clustered sampling.

The practice of culling CWD-positive herds to minimize the risk of spreading the disease has raised some concerns about negative impacts on population viability. However, we did not detect population bottlenecks, and allelic diversity was equal to (or greater than) that documented in other regional studies (Anderson et al. 2002; Blanchong 2003), suggesting that reductions in deer abundance did not result in an observable loss of genetic diversity within the time frame examined. More importantly, as predicted by theoretical studies (Lloyd-Smith et al. 2005) and modelling scenarios (Wasserberg et al. 2009) to date, culling as a management strategy has lowered prevalence in CWD-positive areas in Illinois and minimized its spread to other areas (H.Y. Weng, unpublished data). These are optimistic results in an otherwise rather bleak prognosis on containment and potential eradication of CWD.

Our findings have shown that in heterogeneous landscapes such as Illinois, white-tailed deer populations are maintained by long-distance male dispersal and female philopatry. Profound differences in males and female genetic structure underscore the importance of utilizing several statistical approaches to quantify gene flow at varying spatial scales. With this comparative approach, we were able to make meaningful conclusions about the genders despite vast differences in their movement behaviours. This work contributes to an overall understanding of deer population genetics and wildlife disease while providing a larger context for the comparison of demography, behaviour and genetic tendencies of deer in Midwestern North America. Furthermore, our study has shown that with careful analysis indirect estimates of movement can be
obtained from samples collected through disease surveillance, a finding that offers great potential for future studies examining the interplay between disease and dispersal. To limit spatial expansion of infectious disease outbreaks in cervids, males should be targeted for harvest and surveillance across a broad geographic range as they are readily capable of spreading disease. To limit spatial distance. Funding was provided by IDNR, United States Geological Survey, Federal Aid in Wildlife Restoration Project W-146-R, Illinois Natural History Survey and Environmental Council SURE grant programme.

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