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Genetic assignment tests reveal dispersal of white-tailed deer: implications for chronic wasting disease

Michelle L. Green, Mary Beth Manjerovic, Nohra Mateus-Pinilla,* and Jan Novakofski

Department of Animal Sciences, University of Illinois Urbana–Champaign, 1503 S Maryland Drive, Urbana, IL 61801, USA (MLG, MBM, JN)
Illinois Natural History Survey, University of Illinois Urbana–Champaign, 1816 S Oak Street, Champaign, IL 61820, USA (MLG, MBM, NM-P)

* *Correspondent: nohram@illinois.edu*

Investigating sources of infection for new disease cases is critical to effective disease management. Chronic wasting disease (CWD) was first detected among white-tailed deer (*Odocoileus virginianus*) in Illinois in 2002. Although CWD was focused in northern Illinois, 4 infected deer were sampled in 2011 from locations greater than 100 km south of the disease focus. We used assignment tests (Geneclass2 and Oncor) to determine a likely genetic source location for infected deer. Our baseline data set consisted of 310 deer sampled from 10 locations. From the baseline data set, we determined the most likely genetic source location of 15 CWD-positive and 15 CWD-negative deer. A total of 17–20% back-assigned to their sample location as their most likely genetic source location and the remainder of the animals cross-assigned to another location. The average distance between locations was 41.4 km for Geneclass2 and 43.4 km for Oncor (range 0.0–90.8 km). Distances between source and sampling locations were similar for positive and negative animals. Distances for males were greater than those for females using Oncor, but there was no difference in distance based on age. Because there are few barriers to gene flow for white-tailed deer, managers should reduce movement of deer in CWD-infected areas in an effort to reduce direct and indirect transmission of CWD.

Key words: animal movements, assignment test, dispersal, *Odocoileus virginianus*, prion, transmissible spongiform encephalopathy

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The movement of individual animals, particularly dispersal and migration of infected hosts (Hosseini et al. 2006), influences the spatial spread of directly transmitted diseases (Grenfell et al. 2001; Russell et al. 2004; Hosseini et al. 2006). Directly transmitted diseases move through populations of free-ranging animals by waves (Russell et al. 2004). These waves travel away from the original site of infection and have been explained by the spread of infective "spark" individuals that move from core disease areas to new locations (Grenfell et al. 2001).

Chronic wasting disease (CWD—Williams and Young 1980) is a fatal neurological disease of North American cervids. CWD is a transmissible spongiform encephalopathy that causes the accumulation of abnormally folded cellular prion proteins that ultimately lead to lesions in the brain. As evidenced by the emergence of the disease in recent years, CWD is considered the most contagious member of the prion family (Gilch et al. 2011). Although transmission mechanisms are still not completely understood, infection primarily occurs by horizontal transmission through direct contact (Miller et al. 1998, 2000; Miller and Williams 2003) and indirect exposure to prions in the environment (Miller and Williams 2003; Mathiason et al. 2006; Angers et al. 2009; Haley et al. 2009; Tamgüney et al. 2009). Vertical transmission from mother to fetus also may occur in white-tailed deer (*Odocoileus virginianus*—Nalls et al. 2013), but this transmission route is thought to be rare (Miller and Williams 2003). Clinical signs are not detectable until at least a year following exposure (Williams and Miller 2002). As animals advance through the disease, their infectivity increases and they shed more infectious prions into their environment (Williams and Miller 2002). Because CWD is contagious and invariably fatal, wildlife managers are concerned with limiting prevalence and geographic spread.

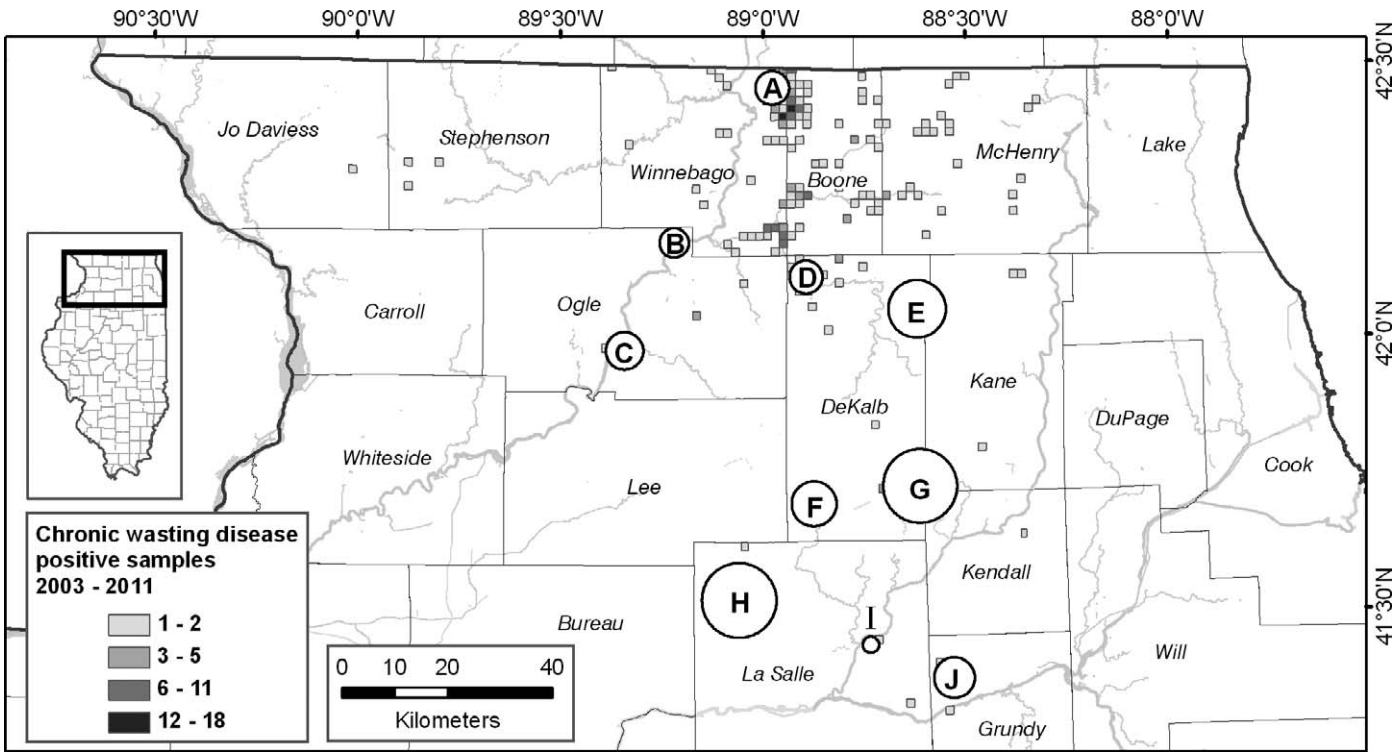

Fig. 1.—The total number of chronic wasting disease (CWD)–positive white-tailed deer (*Odocoileus virginianus*) sampled in Illinois from 2002 to 2013, indicated by gray-shaded boxes. Circles with letters indicate sampling locations for white-tailed deer sampled from 2011 to 2012 for genetic assignment tests. Circle diameter is calculated as standard distance (ArcGIS—Environmental Systems Research Institute [ESRI] 2012) to reflect the relative geographic size of each site. The original spark cases were sampled from locations H (adult female), I (adult female and adult male), and J (adult male) in 2011.

Prevalence of CWD has been attributed at least partially to animal movement (Conner and Miller 2004). Deer exhibit a variety of movement behaviors (see review in Stewart et al. 2011) that contribute to disease transmission and spread. Long-distance and frequent movements are of obvious concern to disease management, but resident deer exhibiting minimal movement within a home range have the potential to severely contaminate their environment through prion shedding (Williams and Miller 2002; Sigurdson and Miller 2003; Mathiason et al. 2006; Angers et al. 2009; Tamgüney et al. 2009; Haley et al. 2011). In areas where infected deer have shed prions into the environment, other deer will be at risk of CWD infection from environmental contamination (Miller et al. 1998). Given the fact that prions persist in the environment for years after initial contamination, infection risk also persists over time (Miller et al. 2004). If infected home ranges have a steady occupancy rate, new occupants are at risk of becoming infected and shedding additional prions into the environment, effectively producing a continuous contribution of prions. Such a state of prion load in the environment not only has the potential to infect animals that move into the area and stay for an extended period of time, but also those that visit the site for a short time and carry the infection to a new location after migration or dispersal events.

In Illinois, wildlife managers have been working to control the spread of CWD since it was first detected in November 2002 (Mateus-Pinilla et al. 2013; Manjerovic et al. 2014). The 1st case, an adult female white-tailed deer, was culled in Boone County (Fig. 1). Since the 1st detection, a disease focus has established along the border of Boone and Winnebago counties. Many of the CWD-infected animals sampled since 2002 were collected in the disease focus but the geographic range of CWD has spread. As of May 2013, CWD-infected deer have been detected in 12 counties (Fig. 1). Wildlife managers in Illinois employ a 2-part management program for disease surveillance and control (Manjerovic et al. 2014). Much of the disease detection and surveillance is accomplished by testing deer culled by recreational hunters. Sampling hunter-harvested deer allows for testing over a large geographic range and increases the chance of identifying infected animals as the disease spreads. In 2011, recreational hunters culled a small number of potential CWD-positive spark cases in La Salle and Grundy counties, far south of the disease focus (approximately 100–125 km [Fig. 1]).

Using genetic assignment tests, we assessed various locations to determine the most likely source location of CWD-infected deer. We were particularly interested in determining the source location of potential CWD spark cases that were sampled in La Salle and Grundy counties and determining whether they originated from the disease focus. However, we also were interested in determining the source locations of several other CWD-infected deer that were

sampled throughout the northern Illinois landscape. Based on the results of the assignment tests, we tested whether the distance between source and sampling locations of infected animals was larger than that of noninfected animals. This project addressed 2 specific objectives: to determine the most likely genetic source location of CWD-infected deer, and to determine whether demographic factors or disease status influenced the distance between genetic assignment locations and sampling locations.

Materials and Methods

Field sampling.—Tissue samples from white-tailed deer were obtained through state of Illinois CWD surveillance and disease-control programs during the fall of 2011 and winter of 2012. The surveillance program focused primarily on collecting samples from deer culled by recreational hunters, whereas the disease management program in Illinois dispatched sharpshooters to locations where CWD-infected animals had been detected. The goal of the disease management program was to reduce deer density on a small geographic scale, thereby limiting disease transmission and spread (Mateus-Pinilla et al. 2013; Manjerovic et al. 2014). All samples used in the current study were tested for the presence of CWD by the Illinois Animal Disease laboratories using the gold standard immunohistochemical examination of retropharyngeal lymph nodes or obex tissue samples, or both, following the National Animal Health Laboratory Network protocol (SOP-PPE-0046; http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/ApprovedSOPList.pdf) using Ventana equipment and antibodies (Ventana Medical Systems, Inc., Tucson, Arizona). We selected 10 sites in northern Illinois as potential source locations of the spark deer (Fig. 1). We selected sites with known relatively high CWD prevalence and geographic locations from which potential disease spread seemed likely based on observations of spread over the past 10 years. Approximately 30 individuals from each of the 10 locations were randomly selected, but because deer sampling does not occur in all areas of the state, some sampling sites required an extension of the geographic range to provide the appropriate sample size. The selected animals from all locations were used to provide the baseline genetic population information necessary for assignment tests. From within the baseline data set, we selected all CWD-positive animals and used specific criteria to identify appropriate CWD-negative deer to function as negative control animals. We determined the age, sex, and cull location of each positive deer and selected a negative control animal for each positive animal by matching all criteria as closely as possible. Age estimates were based on tooth development and wear patterns (Severinghaus 1949). Locations were based on the Public Land Survey System, http://www.nationalatlas.gov/articles/boundaries/a_plss.html, which divided the state of Illinois into a grid of townships (36 miles^2, 129.24 km^2) and township sections (1 miles^2, 2.59 km^2). Illinois Department of Natural Resources biologists used hunter reports to determine sample locations of deer collected through the surveillance program and recorded the locations of deer collected through the disease management program at the time of collection. The CWD-positive deer and their matched controls are referred to as the assignment subset and all genotyped deer are referred to as the baseline data set.

DNA analyses.—Tissue samples were stored in ethanol before DNA extraction. Extractions were completed using the Extract-n-Amp Tissue PCR Kit (Sigma-Aldrich, St. Louis, Missouri). All individuals were genotyped using microsatellite primers designed for white-tailed deer. This panel included markers Eth152 (Steffen et al. 1993); N and Q (Jones et al. 2000); Srcrsp10 (Bhebhe et al. 1994); IGF-1 (Kirkpatrick 1992); OCAM (Fries et al. 1993); RT7, RT9, RT27, and RT30 (Wilson et al. 1997); and BM1225, BM4107, and CSN3 (Bishop et al. 1994). Null alleles were previously found when using CSN3 with Illinois white-tailed deer, and as a result, we selected a redesigned reverse primer for the locus (Kelly et al. 2011). Forward primers were labeled with fluorescent dyes (NED, HEX, and FAM) and fragments were sized on an ABI 3730XL capillary sequencer (Applied Biosystems, Waltham, Massachusetts). Chromatograms were analyzed with Genemapper version 4.0 (Applied Biosystems). We used Micro-checker version 2.2.3 (van Oosterhout et al. 2004) to evaluate the presence of stuttering, large allele drop-out, and null alleles.

Tests for deviation from Hardy–Weinberg equilibrium (overall deviation, heterozygote deficiency, and heterozygote excess) and linkage disequilibrium were carried out using Fisher's exact tests and the Markov chain method (10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch) using Genepop version 4.2 (Raymond and Rousset 1995; Rousset 2008). Allele frequencies, number of alleles per locus, polymorphic information content (Hearne et al. 1992), estimates of null allele frequency, and levels of gene diversity estimated as expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated by Cervus version 3.0 (Marshall et al. 1998; Kalinowski et al. 2007b). Using the baseline data set, we estimated differentiation among locations by calculating F_{ST} in Arlequin version 3.5 (Excoffier and Lischer 2010). We estimated significance tests based on 10,000 random permutations of the data and applied Bonferroni corrections for multiple tests. Input files for Genepop and Arlequin were constructed using Convert version 1.31 (Glaubitz 2004).

Assignment tests.—We used the Bayesian assignment test method implemented in the program GeneClass2 (Piry et al. 2004) and the maximum-likelihood assignment test method implemented in Oncor (Kalinowski et al. 2007a) to determine the most likely genetic source location of individual deer. Because our study area is genetically admixed, we limited our analysis to 1 year to increase the confidence of the genetic assignment tests. Assignment tests require comparison of individuals to baseline genetic information. We used all sampled and genotyped individuals to generate the baseline

Table 1.—Demographic information of 310 white-tailed deer (*Odocoileus virginianus*) sampled in northern Illinois from 2011 to 2012. Each deer was genotyped and used to provide genetic baseline population information for genetic assignment analyses. Location represents the geographic area where animals were sampled. Average age (years) per location was calculated from estimated ages of deer at the time of genetic sampling. CWD = chronic wasting disease.

Location	Average age (*SE*)	Total no.	No. female	No. male	No. CWD positive	(Female : male)	No. CWD negative	(Female : male)
A	2.1 (0.23)	37	22	15	3	(1:2)	34	(21:13)
B	2.3 (0.24)	30	17	13	1	(0:1)	29	(17:12)
C	2.0 (0.21)	28	16	12	0	(0:0)	28	(16:12)
D	2.1 (0.22)	29	15	14	6	(3:3)	23	(12:11)
E	2.0 (0.26)	30	17	13	0	(0:0)	30	(17:13)
F	1.8 (0.18)	31	15	16	0	(0:0)	31	(15:16)
G	1.4 (0.18)	34	15	19	1	(0:1)	33	(15:18)
H	1.9 (0.10)	32	16	16	1	(1:0)	31	(15:16)
I	2.8 (0.50)	31	27	4	2	(1:1)	29	(26:3)
J	2.0 (0.15)	28	20	8	1	(0:1)	27	(20:7)
Total		310	180	130	15	(6:9)	295	(174:121)

data set. In GENECLASS2, we assigned a source location for each deer in the assignment subset using an assignment threshold of 0.05 with Rannala and Mountain (1997) as our criteria for computation. We used the leave-one-out procedure (Efron 1983) and removed the individual being assigned from the reference data set before assignment analysis. In ONCOR, we loaded a reference data set of genotypes from the baseline data set, excluding the assignment subset. We used the positive and negative animals in the assignment subset as the mixture group for analysis and selected the Individual Assignment option to assign each of the animals in the mixture file to a genetic source location.

Distance and direction.—For each animal in the assignment subset, we estimated the distance between the genetic source location and sampling location. The sampling location (township section) of all deer in the study was known. All sampled deer were divided into our 10 baseline locations. We grouped the animals in each location and identified the township sections where all deer within the location had been sampled. We determined the geographic centroid of each location by determining the center of all sampled township sections per location. For each positive deer and the matched negative controls, we measured from the centroid of the most likely source location to the center of the township section where the deer was sampled. To determine whether there was a discernible pattern in the movement of CWD-positive deer compared to their negative counterparts, we estimated the bearing from the most likely source location to the sample location for animals in the assignment subset.

Statistical analyses.—Statistical analyses were carried out using SAS version 9.2 (SAS Institute Inc. 2010). A *t*-test was used to test for differences in distance estimates resulting from assignments generated from GENECLASS2 and ONCOR. Analyzing the assignments from each program individually, we evaluated differences in estimated distance between the most likely source location and the sampling location using a general linear model (Proc GLM) with distance as a dependent variable and disease status (positive or negative), sex, and age (0.5–5 years) as independent response variables. All interactions of variables were included in the initial model. Only predictors showing an association at a significance of 0.05 were included in the final model and considered significant.

Results

Field sampling and DNA analyses.—A total of 310 (average 31 per location) white-tailed deer were genotyped for this study (Table 1). Of these, 58% were female and 42% were male. Fifteen (6 female and 9 male) CWD-positive deer were identified among the baseline data set, including the spark cases. The spark cases (2 females and 2 males) were sampled in locations H, I, and J (Fig. 1). The additional 11 positives were sampled from locations A, B, D, and G.

All markers except RT30 were in Hardy–Weinberg equilibrium. Null alleles also were identified in RT30, but not other markers. Because RT30 did not conform to Hardy–Weinberg expectation, it was removed from all subsequent analyses. No significant linkage between loci was detected after Bonferroni correction. The loci used in our analyses exhibited high levels of polymorphism (12.4 alleles per locus; range 4–18 alleles per locus), with mean observed and expected heterozygosities of 0.75 and 0.79, respectively (Table 2). Estimates of genetic differentiation by location (F_{ST}) ranged from 0.0000 to 0.0192, and 12 of 45 location pairs were different ($P < 0.05$; Table 3). When analyzed by sex, 75% of location pairs were different for females, but only 17% were different for males. All location pairs except B/G, D/I, and D/J were genetically different among females. Only B/G and D/J were different among males.

Assignment tests.—GENECLASS2 and ONCOR agreed on genetic source location assignments for 90% of animals ($n = 30$; Table 4). The programs disagreed on the genetic source location of 1 positive male. GENECLASS2 assigned the individual to location I, whereas ONCOR assigned it to H. Two additional negative control males were assigned to location G by ONCOR but GENECLASS2 assigned the same animals to locations D and F. GENECLASS2 back-assigned 20% of animals to their sample location and ONCOR back-assigned 17%. GENECLASS2 cross-assigned 83.3% of males and 75% of females. ONCOR also

Table 2.—Summary statistics of the microsatellite marker suite used for genetic assignment tests. Statistics based on 310 white-tailed deer (*Odocoileus virginianus*) sampled in northern Illinois in 2011. For each locus, the number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (PIC), and estimates of null allele frequencies are given.

Locus	No. alleles	H_O	H_E	PIC	Null allele frequency
BM1225	10	0.76	0.74	0.70	−0.0130
BM4107	13	0.81	0.82	0.80	0.0085
$CSN3_{(redesigned)}$	4	0.44	0.51	0.40	0.0636
Eth152	16	0.80	0.85	0.83	0.0281
IGF-1	11	0.68	0.66	0.63	−0.0193
N	18	0.84	0.87	0.86	0.0149
OCAM	11	0.80	0.84	0.82	0.0234
Q	18	0.84	0.89	0.88	0.0278
RT27	14	0.88	0.88	0.86	−0.0043
RT7	15	0.75	0.86	0.84	0.0698
RT9	10	0.80	0.81	0.78	0.0072
Srcrsp10	9	0.64	0.74	0.71	0.0726
$\bar{X}$	12.4	0.75	0.79	0.76	0.0233

cross-assigned 75% of females, but the proportion of cross-assigned males increased to 89%. Most animals that cross-assigned (87%) were assigned to a location that was not significantly different from their sample location. Four males (3 positive and 1 negative) cross-assigned to a genetic source location that, according to F_{ST}-values (Table 3), was significantly different from the sample location (Table 4).

Distance and direction.—The average distance between the most likely source location and sample location for all animals in the assignment subset was 41.4 km ($SE = 5.0$ km) in GENECLASS2 and 43.1 km ($SE = 4.7$ km) in ONCOR (Table 5). When all data in the assignment subset were combined, there was no difference in the distance of hypothesized animal movements based on the differing assignments produced by GENECLASS2 and ONCOR ($t_{58} = 0.30$, $P = 0.80$). Distances between sampled and assigned locations were greater for males than females using ONCOR ($F_{1,28} = 4.47$, $P = 0.04$), but not GENECLASS2 ($F_{1,28} = 2.72$, $P = 0.11$). The distances were not different using the assignment tests generated by GENECLASS2. Distance did not differ by age, disease status, or the interactions of those variables regardless of assignment program ($P > 0.05$). Using GENECLASS2, a total of 14 animals (4 females and 10 males) exhibited greater than average distances of 41.4 km (range 43.3–90.8 km) between source and sampling locations. Of those 14 animals, 7 (2 females and 5 males) were CWD positive. Using ONCOR, the same 14 animals plus 1 additional male (4 females and 11 males) exhibited greater than average distances of 43.4 km (range 45.7–90.8 km). Of those 15 animals, 7 were again CWD positive. According to both GENECLASS2 and ONCOR, the longest distances were traveled by males. The longest distance assigned to a female, which was CWD positive, was 64.3 km. A total of 6 animals were assigned to source locations > 68 km (range 68.3–90.8 km) from their sample location. All of these animals were male and 4 of the 6 were positive for CWD. The average distance between source and sampling locations of the original 4 spark deer (2 females and 2 males) was 54.7 km ($SE = 22.0$ km, range 0.0–90.8 km). There was no discernible pattern in the direction of paths from assigned source locations to sample locations (Fig. 2).

Discussion

To design and implement effective disease-control strategies, understanding movement of infected animals across the landscape is critical. The majority of deer in our study cross-assigned to source locations that were different than their sampling location. The average distance between source and sampling locations was approximately 40 km, although we identified distances up to 90 km. Our analysis revealed distances of males between source and sampling locations were larger than those of females, but no relationship was found between distance and age or disease status.

Although deer exhibit a variety of movement types, our reported distances between genetic source and sampling locations fell within the expected dispersal range of Illinois deer (Nixon et al. 1991). Deer typically disperse distances of 4–10 km (DeYoung 2011), but distances can vary considerably (Sparrowe and Springer 1970). Illinois deer disperse 41–49 km on average (Nixon et al. 1991), similar to the average distances we found based on genetic assignment tests.

Table 3.—Matrix of geographic distances (km) and genetic differentiation assessment (F_{ST}-values) of 10 sampling locations for white-tailed deer (*Odocoileus virginianus*) in northern Illinois from 2011 to 2012. Distances reported in the upper right represent the straight-line distance measured from centroid to centroid of each sampling location. F_{ST}-values in the lower left were calculated based on 12 polymorphic microsatellite loci and 310 white-tailed deer. Significant F_{ST}-values shown in boldface type.

Location	A	B	C	D	E	F	G	H	I	J
A	*	37.3	61.3	39.0	53.7	84.9	86.3	104.4	114.9	125.4
B	0.0000	*	24.2	27.7	51.1	60.2	70.3	73.9	90.9	105.1
C	0.0000	0.0010	*	39.8	60.0	49.5	66.0	55.8	77.9	94.3
D	0.0000	0.0012	0.0001	*	23.4	46.1	48.4	67.1	75.9	86.8
E	0.0000	0.0000	0.0000	0.0000	*	44.8	36.0	69.4	68.9	75.3
F	0.0000	0.0012	0.0006	0.0000	0.0000	*	21.8	24.9	30.9	45.4
G	0.0000	**0.0112**	0.0036	0.0000	0.0000	0.0000	*	43.5	33.8	39.6
H	0.0000	**0.0137**	**0.0090**	0.0007	0.0000	0.0000	0.0000	*	28.2	46.5
I	0.0000	**0.0174**	**0.0074**	**0.0070**	0.0000	0.0032	0.0044	**0.0080**	*	18.2
J	0.0000	**0.0192**	**0.0087**	**0.0070**	0.0000	0.0033	0.0032	**0.0092**	**0.0136**	*

Table 4.—Genetic assignment test results of chronic wasting disease (CWD)–positive ($n = 15$) and CWD-negative ($n = 15$) white-tailed deer (*Odocoileus virginianus*) in northern Illinois, sampled from 2011 to 2012. The order of the CWD-positive and CWD-negative deer identification numbers reflect negative control match set pairing (e.g., deer 1146 is the negative control match for deer 1067). Results are reported for 2 programs, Geneclass2 and Oncor. Sample location represents the location where each deer was genetically sampled. Age is the estimated age (years) of the deer at the time of genetic sampling. Assigned locations are the most likely genetic source locations of the deer based on genetic assignment tests. ID = identification; M = male; F = females.

	Deer ID	Sex	Age	Sample location	Geneclass2 assigned location	Geneclass2 −log(L)	Oncor assigned location	Oncor probability
CWD positive	1067	M	1	A	A	14.1	A	0.66
	1014	M	1	A	A	9.9	A	0.68
	1016	F	3	A	C	14.7	C	0.99
	1160[a]	M	2	B	H	14.2	H	0.66
	1019	M	1	D	F	18.0	F	0.60
	1110	M	2	D	C	14.4	C	0.85
	1070	M	3	D	H	16.2	H	0.95
	1078	F	4	D	G	16.1	G	0.56
	1258	F	4	D	C	16.7	C	0.71
	1129	F	5	D	B	16.5	B	0.98
	1096	M	1	G	I	14.6	H	0.52
	1240[b]	F	2	H	G	15.2	G	0.88
	1314[a,b]	M	3	I	B	15.3	B	0.95
	2215[b]	F	3	I	I	16.7	I	0.82
	2004[a,b]	M	2	J	C	23.4	C	0.84
CWD negative	1146	M	1	A	B	14.7	B	0.94
	1159	M	1	A	G	14.3	G	0.80
	1085	F	3	A	B	13.3	B	0.77
	1111[a]	M	2	B	G	15.6	G	0.95
	1023	M	1	D	F	16.7	F	0.99
	1119	M	2	D	G	17.4	G	0.87
	1099	M	2	D	D	12.9	G	0.92
	1206	F	4	D	A	11.8	A	0.91
	1087	F	3	D	G	11.6	G	0.98
	1212	F	4	D	F	21.0	G	0.45
	1024	M	1	G	C	18.0	C	0.85
	2443[c]	F	2	H	H	15.7	H	0.93
	1306[c]	M	1	I	G	17.9	G	0.84
	2182[c]	F	3	I	I	17.4	I	1.00
	2088[c]	M	2	J	G	13.8	G	0.84

[a] Animals whose source and sample locations were genetically different according to F_{ST}.

[b] An original spark deer.

[c] Chronic wasting disease–negative animals matched to the original spark deer by sex, age, and sampling location.

The proportion of deer that disperse commonly varies with sex (Sparrowe and Springer 1970). Dispersal is usually biased toward males, although females also disperse (Nelson and Mech 1992; Nelson 1993; Rosenberry et al. 1999; Purdue et al. 2000; DeYoung 2011). In Illinois, radiotelemetry and tagging studies suggested a relatively large proportion of female fawns disperse with estimates ranging from 21% to 50% compared to male estimates of 56–78% (Nixon et al. 1991, 1993, 1994, 2007). Our results agree with the dispersal pattern seen among fawns, which is thought to be the largest dispersing cohort. Although high female dispersal rates complicate efforts to contain CWD outbreaks (Nixon et al. 2007), the fact that both sexes disperse in Illinois indicates that a large proportion of each fawn cohort and a smaller, but substantial, proportion of yearling and adult cohorts can potentially contribute to disease spread via dispersal behavior.

Genetic assignment tests indicated more than 80% of males and 75% of females were cross-assigned based on deer collected by both hunters and sharpshooters. A small proportion of hunters may not be truthful about the location of a kill for a variety of reasons (e.g., confusion, secrecy, or illegal activity). In a well-known Illinois poaching case, the defendant reported a false location within 16 miles of the true location (Kiernan 2011). Therefore, we expect inaccurate locations to be close to the true location and given the geographic scale of our study, inaccuracy from hunters is not likely to affect our result. Our results suggest a higher proportion of dispersal compared to radiotelemetry studies (Nixon et al. 1991, 1994, 2007). Estimates of dispersal based on observational field methods are often lower than dispersal estimates generated from genetic data. In fact, comparative studies of other vertebrates indicate that both the mean and variance of dispersal distances may be much larger than would be suspected based on observational studies alone because of limits in sample size, area, and generations observed (Koenig et al. 1996). Given the known difference in methods, it is not surprising that our dispersal rate was higher than those estimated by radiotelemetry and tracking studies.

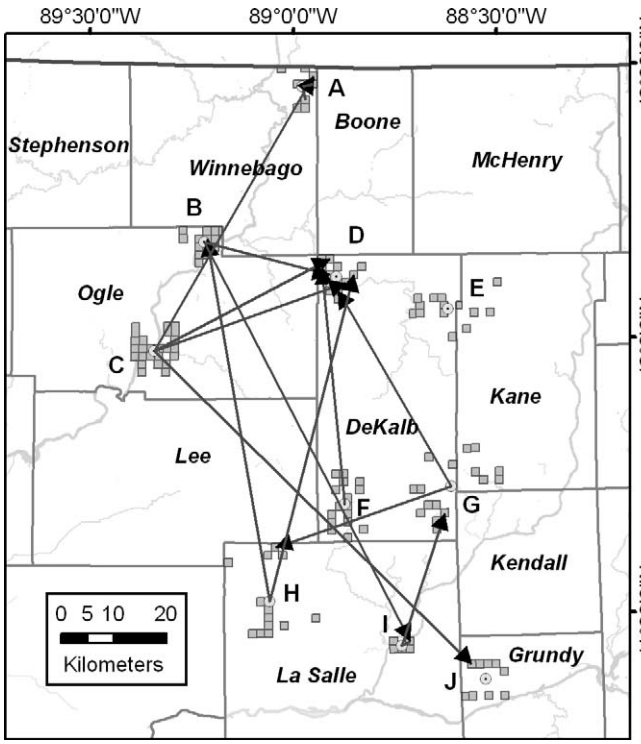

Fig. 2.—Map showing the most likely genetic source locations and sampling locations of chronic wasting disease–positive white-tailed deer (*Odocoileus virginianus*) in Illinois based on genetic assignment tests in Geneclass2. Each line represents 1 individual. Lines were drawn from the centroid of the most likely source location to the centroid of the township section where the individual was sampled. Lines terminate in an arrowhead at the sampling location. The arrowhead provides a visual representation of the hypothesized movement of an animal from its source location to the township section where it was later sampled.

Documented large-scale movement of deer out of CWD-infected areas raises legitimate concerns regarding disease spread. Oyer et al. (2007) tracked a female that dispersed a straight-line distance of 98 km out of the Wisconsin CWD area. Several additional long-distance dispersal events have been reported in white-tailed deer. Nixon et al. (1991) identified 2 females that dispersed or relocated to new home ranges approximately 70 km from their original sites. In Minnesota, female dispersal distances of 77, 168, and 205 km were recorded (Nelson and Mech 1992; Nelson 1993; Brinkman et al. 2005). Similarly, in South Dakota female deer moved distances of 163 and 225 km, and males moved 204 and 213 km (Sparrowe and Springer 1970; Kernohan et al. 1994). The small number of long-distance movements identified by tracking methods may provide a low expectation of disease spread that can lead to reduced concern among stakeholders. On the other hand, genetic methods have indicated extensive dispersal of male white-tailed deer in northern Illinois at scales of < 100 km and > 100 km (Kelly et al. 2010). Admixture proportions suggested that dispersal events ≤ 300 km occur and that CWD could spread across the landscape through such long-distance movements (Kelly et al. 2010). Our results agreed with those of Kelly et al. (2010) in that genetic tools identified large dispersal distances and high levels of deer movement between locations in Illinois, indicating a more realistic estimate of deer dispersal and the risk of CWD spread as a result of large-scale movement.

The pathway by which infected animals came to be in La Salle and Grundy counties (Fig. 1) remains unknown but 2 hypotheses exist. The deer may have become infected with CWD in another location (e.g., the disease focus in northern Illinois) and moved to a new area, bringing the infection to new geographic locations and qualifying those animals as true spark cases. Alternatively, the animals may have been local residents that were infected with CWD in their home environment. This 2nd explanation assumes other animals brought prions into the area, and a series of intermediate disease transmissions may have existed between the disease focus and the new locations. Genetic assignment tests are unable to determine whether individuals stayed in one location for their entire life, or if they made any migratory, transient (movement among 2 or more home ranges independent of seasonal cues), or exploratory movements (occasional, temporary movements outside of the home range; movement definitions from Skuldt et al. [2008]). Exploratory movements were common among adult deer, whereas transient and migratory movements were less frequent

Table 5.—Mean estimated distances (km) between the most likely genetic source location and sample location for chronic wasting disease (CWD)–positive and CWD-negative white-tailed deer (*Odocoileus virginianus*) sampled in northern Illinois from 2011 to 2012. Distance estimates are based on genetic assignment tests from Geneclass2 and Oncor.

		Geneclass2			Oncor		
	n	Distance	*SE*	Range	Distance	*SE*	Range
All animals	30	41.4	5.0	0.0–90.8	43.4	4.7	0.0–90.8
Female	12	31.6	6.1	0.0–64.3	31.8	6.1	0.0–64.3
Male	18	47.9	6.9	0.0–90.8	51.1	6.2	0.0–90.8
CWD positive	15	43.7	7.8	0.0–90.8	44.5	7.7	0.0–90.8
Female	6	35.8	8.8	0.0–64.3	35.8	8.8	0.0–64.3
Male	9	48.9	11.7	0.0–90.8	50.2	11.4	0.0–90.8
CWD negative	15	39.0	6.4	0.0–82.1	42.3	5.7	0.0–82.1
Female	6	27.4	8.9	0.0–47.9	27.8	9.0	0.0–47.9
Male	9	46.8	8.1	0.0–82.1	52.0	5.7	33.4–82.1

but still documented in the population (Nixon et al. 1991; Skuldt et al. 2008). The previously mentioned Wisconsin deer that traveled a straight-line distance of 98 km out of the CWD area actually undertook a series of dispersal, exploratory, and transient movements resulting in a cumulative distance of 462 km traveled in just over a year (Oyer et al. 2007). Because genetic assignment tests cannot elucidate these intermediate movements, the actual distance deer travel during dispersal and relocation events is likely underestimated by genetic methods. Disease managers should carefully consider this underestimate of intermediate movements when using genetic tests to develop management protocols because intermediate movements may increase risk of disease spread as a result of direct animal contact and environmental contamination or exposure to prions.

By using reported distances between source and sample locations to categorize our results as dispersal, examination of our data suggests that many deer disperse longer than the average distance reported by tagging or radiotelemetry observations (Nixon et al. 1991), which is consistent with the genetic findings of Kelly et al. (2010). Both long-distance movements and shorter-distance movements are cause for concern regarding CWD. Because genetic assignment tests can provide an estimate of movement and specific source information for a subset of selected individuals and tracking methods can reveal detailed transient or exploratory movements, we recommend managers and biologists combine molecular and tracking information to compile a more complete understanding of deer movement. However, given the infectivity of CWD-positive deer and environmental risk of contamination, we recommend that radiotelemetry studies be completed in disease-free locations, or be completed before infection. After the disease is found in an area, research should shift exclusively to molecular methods to focus management efforts on reducing deer movement and reducing the risk of infection to additional animals. Given long-distance dispersal, admixture, and potential disease spread, we recommend management actions to reduce both small- and large-scale movement in and out of the known CWD-infected areas in Illinois. Although difficult, such management may slow the spread of CWD across the landscape by inhibiting deer movement, decreasing environmental loads of the pathogen, and offering some protection to geographic areas that are currently disease free.

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