THE EXPANDING DISTRIBUTION OF IXODES SCAPULARIS AND ASSOCIATED PATHOGENS IN THE CHICAGO, IL, METROPOLITAN AREA

BY

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THESIS

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ABSTRACT

The geographic distribution of Lyme disease in the United States has increased considerably since the first description of the illness in the 1970s. The primary vector of Lyme disease, the tick *Ixodes scapularis* (Acari: Ixodidae), has expanded its range concurrently with the disease including into urban landscapes. To investigate landscape factors that may influence the colonization of *I. scapularis* and its associated pathogens in an urban ecosystem, 45 sites were sampled along three transects spanning the urban-to-rural human land use gradient in the Chicago, Illinois, metropolitan area. I collected four species of ticks (88% were *I. scapularis)* which exhibited variable infection rates for six pathogens, including *Borrelia burgdorferi*, the causative agent of Lyme disease, and *B. lonestari* and *B. miyamotoi*, both reported for the first time in Illinois. Logistic regression modeling indicated the presence of *I. scapularis* was positively correlated with forest land cover and negatively correlated with developed land cover, while the presence of *B. burgdorferi* was positively correlated with forest land cover. Neither the presence of the tick or the pathogen were correlated with distance to the nearest major river way. This study suggests that the range of *I. scapularis* and its pathogens have expanded in the Chicago metropolitan area since previous studies were conducted, including into forested urban areas near to the urban core. As tick and pathogen continue to colonize new areas, active monitoring and increased public education will be needed to protect vulnerable human populations.

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Introduction

Infectious diseases that afflict human health are often imbedded within wildlife communities, their transmission dynamics governed by complex ecological interactions. Disease systems involving transmission by arthropods are particularly complex, and many vector-borne diseases are currently emerging or re-emerging (e.g., expanding in distribution or host range). For example, Dengue fever is spread by *Aedes aegypti* mosquitoes and has begun to emerge in areas where not previously present, such as in the Caribbean islands and southern Florida (Gubler and Clark 1995). Chikungunya virus re-emerged in Lamu, Kenya in 2004 and has been spreading east, infecting millions (Powers and Logue 2007). However, even for infectious diseases for which the ecology and transmission dynamics are well understood, there remain many challenges to prevention and control. The ecology of Lyme disease is arguably the best understood of any vector-borne disease, yet infection rates remain high with over 30,000 confirmed and 300,000 estimated cases each year in the United States (CDC 2013a). Human incidence of Lyme disease in the U.S.is focused in two areas, the Northeast and the upper Midwest. Since the discovery of Lyme disease in the early 1970s, these hotspots have continued to expand in range, placing millions of people at risk. While rarely fatal, this disease can cause long-term damage to the joints, nerves, and brain if left untreated (Steere et al. 1979, Reik et al. 1979, Steere et al. 1980).

The black-legged tick, *Ixodes scapularis* (Say), is the primary vector to humans of the bacterium *Borrelia burgdorferi*, the etiological agent of Lyme disease in the U.S. While the distribution of *I. scapularis* in the Midwest was historically centered in Minnesota and Wisconsin, it has expanded greatly in distribution over the last several decades (Dennis et al. 1998, Duik-Wasser et al. 2012). This expanding distribution is likely facilitated by long-distance movements of important wildlife hosts that provide blood meals for different life stages of the tick. For example, larval and nymphal life-stage ticks may feed on migratory birds (Ogden & Lindsay 2008), and adult ticks often feed on white-tailed deer, *Odocoileus virginianus*, populations of which have been expanding (Rand et al. 2003). The spread of *I. scapularis* is cause for concern, but the consequences are only epidemiologically significant if there is a corresponding increase in the distribution of the pathogen. Human incidence of Lyme disease has expanded concurrently

(CDC 2013a), suggesting the spread of *I. scapularis* into new regions may carry significant health consequences for local human populations.

Ixodes scapularis was first reported in the state of Illinois in 1988 (Bouseman et al. 1990), and *I. scapularis* and *B. burgdorferi* were reported in the Chicago region as early as 2006 (Jobe et al. 2006). Since then, both tick and pathogen have spread throughout a considerable portion of the Chicago metropolitan area, particularly in the state forest preserve system which provides the deciduous forest habitats and associated wildlife hosts necessary for the tick and pathogen to complete their life cycles (Rydzewski et al 2012). Forested ecosystems of Chicago are characterized by a high degree of habitat fragmentation, a phenomenon known to significantly alter the diversity and community composition of wildlife (Rosenblatt et al. 1999, Debinski and Holt 2000). Species that are more resilient to habitat loss and fragmentation can increase in abundance in urban landscapes, including important wildlife hosts for *I. scapularis* and *B. burgdorferi*. For example, the white-footed mouse, *Peromyscus leucopus* and the eastern chipmunk, *Tamias striatus*, are known to thrive in small forest fragments (Nupp and Swihart1996, 1998), and habitat fragmentation previously has been shown to increase Lyme disease risk (Allan et al. 2003, Brownstein et al. 2005).

The distribution of *I. scapularis* and *B. burgdorferi* are likely influenced by landscape features of the urban-to-rural human land-use gradient around Chicago. *Ixodes scapularis* is a forest-obligate species (Maupin et al. 1991), and many of the important wildlife hosts for both the tick and pathogen are positively associated with forest cover. Forested areas may also function as habitat corridors for wildlife movements into developed areas due to their association with waterways in developed landscapes (Beier and Noss 1998, Gillies and St. Clair 2008). I therefore conducted a field and laboratory study to test the following two hypotheses: 1) the occurrence of *I. scapularis* and *B. burgdorferi* at each site would be positively correlated with the site's proximity to water, and 2) the occurrence of the tick and pathogen would be negatively correlated with developed land cover and positively correlated with forested land cover. I tested these hypotheses through a combination of field surveys for *I. scapularis*, molecular analyses to determine the presence of *B. burgdorferi* and other pathogens in captured ticks, and spatial and statistical analysis.

Materials and Methods

Study Organism:

Ixodes scapularis is a cold tolerant tick with three life stages active during different times of year (Vandyk et al. 1996). In the Midwest, larval *I. scapularis* are active late summer, the nymph life-stage is active mid-summer, and adult activity typically peaks during early spring and late fall (Gatewood et al. 2009). Nymphs are the most epidemiologically significant life-stage due to their mid-summer peak in abundance when humans are active in the outdoors and small size which makes them difficult to detect. *Ixodes scapularis* typically takes up the Lyme pathogen in a juvenile life stage while feeding on a reservoir competent and infected host such as *P. leucopus*. Adult ticks typically feed on large-bodied hosts such as *O. virginianus*, where adult males and females will locate one another and mate. Therefore, wildlife hosts such as *O. virginianus* are critically important to the completion of the tick life cycle, while hosts such as *P. leucopus* are critically important to the completion of the pathogen life cycle.

Site Selection:

Field sites were selected across an urban-to-rural human land-use gradient in the greater Chicago area. Although the urban core is primarily developed, the rural and suburban habitats are primarily low- and medium-density human housing and agriculture. Forested habitat remains a minority of the land-use across the urban-to-rural land-use gradient, with only 14% of the study area being forested. Thus this study offers the opportunity to understand the influence of surrounding land-use types on tick and pathogen occurrence in a recently invaded landscape. Forested areas in the urban core typically consist of parks and cemeteries, and gradually transition to forest preserves and parks toward the rural portion of the land-use gradient.

This study utilized a network of sites established for an urban wildlife ecology project of the Urban Wildlife Institute at the Lincoln Park Zoo. Three transects span the urban-to-rural land-use gradient and include over one hundred cemeteries, parks and natural areas (hereafter ‘sites’). To determine the locations of these sites, transects were divided into 10 equal sections and all green spaces within 1km of the transect lines were identified. Up to four green spaces were selected within each section for sampling. If the LPZ was not able to get permission to sample at a site, a

new site was selected for sampling. ArcMap was used to randomly choose a point within each site where sampling would occur, unless site owners or managers preferred a different location. All three transects extend from the urban core of Chicago and extend 50km north, west, and southwest. A subset of 45 sites were selected for this study based on the presence of forest cover and variation in land use types from the surrounding landscape. Ticks were sampled at one or two locations at each site.

Field Collection:

Questing ticks were sampled from the 45 sites throughout Chicago once-twice per season from 2011-2014 (Table 1). Additional visits at four sites were made by Tom Velat of the DuPage County Forest Preserve District. Weather and logistical constraints limited the number of visits in 2011 and 2012. Ticks were collected by dragging a 1 m^2 white corduroy cloth along the ground and over vegetation (Schulze et al. 1997). Captured ticks were removed from the drag cloth and from the protective suit on the observer's body and placed in 70% ethanol for subsequent identification and molecular analysis. Due to considerable variation in vegetation characteristics between sites, sampling efforts were standardized between sites by recording the time spent drag-sampling for ticks (reported as ticks collected per minute of sampling). Consistent drag transects of fixed distances would not have been feasible at many sites due to variable understory plant density and the physical constraints of small, urban sites. The seasonal timing of the drag sampling was determined by the peaks in activity of different life stages of *I. scapularis* (Gatewood et al. 2009). Sampling on individual days was constrained to suitable climatic conditions for *I. scapularis* nymph and adult questing activity (Schulze et al. 2001). An average drag time of 60 minutes per site was performed in 2011 and 2012, 43 minutes per site in 2013 but with two visits per site, and 19 minutes per site in 2014 and again with two visits per site (Table 1). Less drag sampling was performed in 2014 due to extended periods of unfavorable sampling conditions. Coordinates were recorded for each location where ticks were sampled using a handheld GPS unit (Garmin Rhino 530HCx).

Laboratory Methods:

Both nymph and adult specimens were identified to species using a dissecting microscope (Sonenshine 1979). A total of 205 *I. scapularis* (131 adults and 74 nymphs) and 7 *I. dentatus*

nymphs were processed for pathogen identification by a combination of polymerase chain reaction (PCR) and reverse line blot (RLB) hybridization. DNA was extracted as in Hamer et al. (2001) with the following modifications: ticks were pulverized using a Tissue Lyser II (Qiagen, Valencia, CA) in80 µl (nymphs) or 100 µl (adults) of 5% chelex, incubated for 20 min at 56^{o}C, boiled for 8 min, cooled on ice, then centrifuged at 4000xg for 6 min.

Phusion High Fidelity DNA Polymerase (New England Biolabs; Ipswich, MA) was used in all polymerase chain reaction (PCR assays; additional $MgCl_2$ (4.5mM final concentration) was used due to the presence of residual chelex in the lysates. Amplification occurred in a Veriti Applied Biosystems thermocycler (Life Technologies; Grand Island, NY) with an initial denaturation of 98^{o}C for 30s; followed by denaturation at 98^{o}C for 10s, annealing starting at 60^{o}C then decreasing by 1^{o}C per cycle to 53^{o}C and continuing at this temperature for an additional 39 cycles with extension in each cycle at 72^{o}C for 1 min, followed by a final extension at 72^{o}C for 7 min.

For the 2011 and 2012 samples, bacterial DNA was amplified in a multiplex PCR containing two sets of primers obtained from Integrated DNA Technologies (IDT; Coralville, Iowa). Universal primers 0206 and 0209 from Pichon et al. (2003) were used to amplify a portion of the 16S rDNA. Primers 23SN2 and 5SCB described by Rijpkema et al. (1995) were used to amplify the 23S-5S intergenic spacer of the *B. burgdorferi* sensu lato complex. For the 2013 samples, ompA (primers: RompAF, RompAR) and ompB (primers: ROmpBF, ROmpBR) were added to the protocol for improved detection of *Rickettsia* sp. OmpA and ompB were amplified as a separate multiplex PCR with both multiplex (rRNA and ompA/B) products added together for the RLB assays. Primers 0209, 5SCB, ompA and ompB had a 5'-biotin label to enable amplicons in the RLB assay. Each set of amplifications contained at least one positive and one negative control (Allan et al. 2010). Samples positive for *Borrelia burgdorferi* sensu lato or sensu stricto were confirmed by sequencing. For the RLB analysis, 20 previously published probes and 23 new probes were utilized (Allan et al. 2010, Fredericks et al. In Review).

To confirm the identity of pathogens detected by RLB, DNA from the tick lysate was amplified using a modified protocol from Clark et al. (2005). PCR component concentrations were the

same as in the multiplex PCR for the RLB assay except this nested PCR used 2.0 mM $MgCl_2$. The outer PCR (primers: FlaBOF/FlaBOR) used 5 µl tick lysate and the following protocol: initial denaturation at 98°C for 30s; 40 cycles of 98°C for 10s, annealing at 52^{o}C for 30s, extension at 72°C for 45s; and a final extension at 72°C for 7 min. The nested PCR (primers: FlaBIF/FlaBIR) used 2.5 µl gel purified outer PCR product under the same conditions as the first outer PCR, but with annealing at 55^{o}C, and was performed twice. After each round of amplification, PCR products were gel extracted using either the QIAEX II Gel Extraction kit (Qiagen) (2011 – 2012 samples) or 1.5 µl gelase (2013 – 2014 samples), following the manufacturer's protocol. PCR products were sequenced in both directions (primers: FlaBIF and FlaBIR) by the University of Illinois Core Sequencing Facility using Sanger DNA Sequencing and analyzed on an Applied Biosystems 3730xl automated sequencer with 50cm capillary arrays. Sequence editing and analysis was performed using Geneious (Biomatters Ltd.; Aukland, New Zealand) and the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov/blast/Blast.cgi). For each pathogen targeted for detection, the genes sequenced were (pathogen: gene): *B. burgdorferi* sensu lato: flagellin B; *B. lonestari*: flagellin B with *B. lonestari* primers; *B. miyamotoi*: 16S-23S intergenic spacer region (IGS); *Rickettsia* spp.: ompA; *Francisella tularensis*: 16SrRNA gene.

Spatial Analysis:

The latitude and longitude of each sampling location within each site were collected using a handheld GPS and entered into ArcMap (ESRI, 10.2.1). A land-use/land cover (LULC) database was downloaded from the USGS Gap Analysis Program (http://gapanalysis.usgs.gov/gaplandcover/data/) and sample locations were overlaid. Buffers with radii of 500m, 1000m, and 1500m were created around the centroid of each sample location. These three buffer sizes were assigned because I had no *a priori* expectation as to the effect of buffer size on the relationship between land cover and tick and pathogen presence or absence. Forest and developed land cover were quantified within each buffer class using the 'Clip' function within ArcMap (Table 2). Forest land cover included the cover types: deciduous forest, evergreen forest, and mixed forest. Developed land cover included the cover types: open space, low development, medium development, and high development. The percentage of overlap among buffers of each radius also was calculated. Mean distances between centroids and

standard deviation of these distances were also calculated in ArcMap. To determine distance to nearest major riverway, the recorded GPS points were placed in Google Earth (GeoBasis) and the 'Ruler' tool was used to measure from point to the nearest river edge. River size was determined based upon the assigned Strahler value (Pierson et al. 2008), which within the sampled area ranged from one to four depending on the number of tributaries associated with each river size class.

Statistical Analysis:

We used multiple logistic regressions to determine if the presence of *I. scapularis* or *B. burgdorferi* were correlated with landscape variables. Predictor variables included distance to the nearest major river and percent forest cover and percent developed cover within 500m, 1000m, and 1500m buffers. Goodness of model fit was compared by Akaike Information Criterion (AIC) scores; model fit was considered significantly improved if the score was lower than other candidate models by more than 2. Statistical analyses were performed in Systat version 13.

Results

Tick Distribution and Abundance:

Four tick species were collected across the study area: *I. scapularis* (88% of total), *Dermacentor variabilis* (9%), *I. dentatus* (2%), and *Haemaphysalis leporispalustris* (1%). *Ixodes scapularis* nymphs were detected at 53% of sites, while *I. scapularis* adults were detected at 23% of sites. Abundance and diversity of both nymphs (Figure 1a) and adults (Figure 1b) varied across the region, with the greatest abundance of *I. scapularis* observed in the southern and southwestern study sites. Nymph life stage abundance ranged from 0-0.28 nymphs/minute collected across all sites, while adult abundance ranged from 0-0.43 adults/minute collected across all sites.

Pathogen Diversity and Prevalence:

Six *I. scapularis*-transmitted bacterial pathogens were detected across the region: *B. burgdorferi* (23% of sites), *B. miyamotoi* (10% of sites), *Anaplasma phagocytophilum* (8% of sites), *B. lonestari* (3% of sites), an unknown *Borrelia* spp. (3% of sites), and *Francisella tularensis* (3% of sites). Pathogen prevalence and diversity in both nymphs (Figure 2a) and adults (Figure 2b) varied across the region. Prevalence of *B. burgdorferi*, the focal pathogen of this study, ranged from 0-30% in nymphs collected across all sites and 0-100% in adults collected across all sites.

Spatial Analysis:

Land cover varied considerably among sites and buffer classes. Forest cover ranged from 0-65% for all sites with a buffer of 500m, 0-47% for all sites with a buffer of 1000m, and 0-39% for all sites with a buffer of 1500m. Developed cover ranged from 0-100% for all sites with a buffer of 500m, 10-100% for all sites with a buffer of 1000m, and 22-100% for all sites with a buffer of 1500m. Buffers of different radii also had different degrees of overlap. Overlap between buffers at 500m was 0%, overlap between buffers at 1000m was 5.8% and overlap between buffers at 1500m was 10.6%. Distance from sample location to nearest river way varied from 28.97 to 5923.09m.

Logistic Regression Models:

Logistic regression models were used to consider the effects of distance to nearest major river and percent forest cover and percent developed cover within 500m, 1000m, and 1500m buffers on the presence of *I. scapularis* or *B. burgdorferi*. The model for *I. scapularis* presence with forest and developed cover buffers of 1000m offered significantly better model fit than the candidate models with 500m or 1500m buffers (Table 3). The model for *B. burgdorferi* presence with a forest and developed cover buffer of 500m offered significantly better model fit than the candidate model with the 1000m buffer, and both offered significantly better model fit than the candidate model with the 1500m buffer (Table 3). As the models for *B. burgdorferi* presence with buffers of 500m and 1000m offered qualitatively similar results, for consistency only the models for *I. scapularis* and *B. burgdorferi* presence with the buffers of 1000m are presented. Presence of *I. scapularis* was significantly, positively correlated with forest cover ($Z = -2.080$, $p = 0.038$), significantly, negatively correlated with developed cover ($Z = -2.526$, $p = 0.012$), but not with distance to a major river ($Z = -0.624$, $p = 0.533$; Table 4). Presence of *B. burgdorferi* was significantly, positively correlated with forest cover ($Z = -2.10$, $p = 0.036$) but not with developed cover ($Z = -1.377$, $p = 0.169$) or distance to a major river ($Z = -0.073$, $p = 0.942$; Table 5).

Discussion

The Chicago region presents an opportunity to investigate the ecological consequences of human ecosystem alteration for the establishment of an infectious disease in an urban ecosystem. Here, I examined the hypotheses of whether distance to major river ways and distribution of land cover types (i.e., forest and developed land covers) influenced the likelihood of detecting the components of the Lyme disease system. Habitat fragmentation previously has been shown to lead to an increase in Lyme disease risk (Allan et al. 2003) through ecological changes that result in decreased diversity and increased abundance of key hosts (LoGiudice et al. 2008). In addition to these ecological changes, the edge habitats created by forest fragmentation can create locations where humans and ticks are more likely to come into contact, such as forest edges, further increasing the chance of Lyme disease exposure (Falco and Fish 1989). However, it remains poorly understood how variation in human land use can influence the establishment of *I. scapularis* and *B. burgdorferi* in new regions.

In this study, distance between a sampled site and the nearest major river way was not predictive of the presence of either the tick or pathogen. These results were unexpected because the invasion of *I. scapularis* and *B. burgdorferi* at larger spatial scales appear to follow major river ways in the Midwestern U.S. (Pawlikowski et al., In Preparation). However, the prevalence of forest and developed land cover were positively and negatively correlated, respectively, with the presence of *I. scapularis*, and forest cover was positively correlated with the presence of *B. burgdorferi*. These positive relationships with forest cover were expected because this is the habitat in which *I. scapularis* acquires suitable hosts, many of which are the same species to which *B. burgdorferi* is adapted. However, this is one of the most urban landscapes in which such a study has been conducted and it suggests there may be thresholds in forest and developed land cover, below and above which, respectively, the tick or pathogen may not establish. For example, in this study *B. burgdorferi* was not detected from any site with less than 7% forest cover based upon a 1000m buffer radius. There were 12 such sites in this study and these sites likely are not able to sustain sufficiently large populations of reservoir hosts to maintain *B. burgdorferi*.

While the focal pathogen for this study was *B. burgdorferi*, there were several other pathogens detected in collected ticks: *A. phagocytophilum*, *B. lonestari*, *B. miyamotoi*, an undescribed *Borrelia* species, and *F. tularensis*. *Borrelia lonestari*, putatively a causative agent of Southern Tick Associated Rash Illness (STARI), likely is vectored by *Amblyomma americanum* (Barbour et al 1996). STARI is predominantly diagnosed in the southeastern U.S. and has not been detected in Illinois previously. Symptoms are similar to Lyme disease, so this disease can be considered a risk to human health. *Borrelia miyamotoi* is found both in the western (Mun et al. 2006) and eastern U.S. (Scoles et al. 2001), and also has not been reported from Illinois previously. Rather than causing Lyme-like symptoms, *B. miyamotoi* causes relapsing fever and also should be considered a significant risk to human health. The unknown *Borrelia* species cannot be evaluated for human health risk. *Anaplasma phagocytophilum*, the causative agent of anaplasmosis, has been detected in Illinois previously, though it is a rare disease that infects fewer than 1 in 1,000,000 people (CDC 2013b). Anaplasmosis is considered a minor risk to human health. *Francisella tularensis*, the causative agent of tularemia, is rare in Illinois, with five or fewer cases of tularemia diagnosed each year (IDPH 2015). This pathogen has not been detected in Chicago previously, nor has it been detected previously in *I. scapularis*. Tularemia is considered to be a high risk to human health.

Several tick species were found during sampling for *I. scapularis*, including: *I. dentatus*, *D. variabilis*, and *H. leporispalustris*. *Ixodes dentatus* can be found on small mammals and birds (Sonenshine and Stout 1970, Battaly et al. 1987). It is active from March to November and is considered a poor vector for both *B. burgdorferi* and *Rickettsia ricketsii* (Anderson et al 1989). However, because it rarely bites humans, it is considered important only as an enzootic vector. *Dermacentor variabilis* is most commonly found on mammals (Sonenshine and Stout 1970). It is active from April to September (Conlon and Rockett 1982) and can serve as a vector for *R. rickettsii* (Sonenshine and Stout 1970). While studies have shown that *D. variabilis* can harbor *B. burgdorferi*, it is considered to be a poor vector (Piesman and Sinsky 1988). *Haemaphysalis leporispalustris* is most commonly found on rabbits, but can also be found on small birds (Sonenshine and Stout 1970). Humans are rarely used as hosts. It is active between May and October (Sonenshine and Stout 1970, Kollars and Oliver 2003) and can serve as an enzootic vector of *F. tularensis* in rabbit populations (Sonenshine 1979).

Several previous studies have examined the distribution of the Lyme disease system in the Chicago region. Jobe et al.(2006)detected *I. scapularis* at two out of 10 sites sampled in DuPage County and one out of three sites sampled in Cook County. Collected ticks were tested for *B. burgdorferi*, but fewer than 5% of ticks tested positive. In an effort to better detect the presence of *B. burgdorferi*, Jobe et al. (2007) further collected *I. scapularis* from one site in DuPage County, two sites in Cook County, and four sites in Lake County. Collected adult *I. scapularis* were tested for *B. burgdorferi*, and 37% of 107 tested ticks were positive. In a previous study comparable to this one, Rydzewski et al. (2012) sampled 32 sites in Cook, DuPage, Lake and McHenry counties in 2008 and 2009 and found *I. scapularis* nymphs and/or adults at 17 of these sites. Ticks were not tested for pathogens. Thus when comparing to this previous literature, the results of this study show that the distribution of *I. scapularis* in the Chicago, IL, metropolitan area is greater than documented previously, with nymphs and/or adults detected at 22 of 40 sites sampled. I found *I. scapularis* populations to be most abundant along the western and southwestern transects of this study, while comparatively rare along the northern transect. Some sites at which *I. scapularis* was newly detected in this study are closer to the urban center of Chicago. The detected presence of *B. burgdorferi* at nearly a fourth of all sites sampled suggest the Lyme disease system has become widespread since the first report in the Chicago area only nine year ago.

Both *I. scapularis* and *B. burgdorferi* likely will expand in distribution in the future due to the presence of suitable habitat even within highly fragmented urban areas with large amounts of developed land cover. Factors influencing the invasion ecology of *I. scapularis* and *B. burgdorferi* will need to be further studied to determine areas most at risk to colonization and invasion by the Lyme disease system. Presence and identification of confirmed and potential hosts for larval and nymphal *I. scapularis* in fragmented ecosystems close to urban cores should be evaluated, as should the abundance of important wildlife hosts for adult ticks such as *O. virginianus*. The influence of distance to water also merits further study, not only distance to river ways, but also distance to ponds and lakes, especially those that serve as roosts or stopover sites for migratory birds. As Lyme disease and other tick-borne pathogens expand into new regions, urban areas must be included in the landscapes considered at risk for colonization by

ticks and the pathogens they vector that imperil human health. Here I show that the predominantly urban landscape of a large metropolitan area, such as Chicago, has the potential to harbor vector ticks and a diversity of pathogens, and natural corridors may serve as dispersal routes for *I. scapularis* into increasingly developed landscapes.

Figures and Tables

Figure 1a. Abundance of nymphal *Ixodes scapularis* and other tick species across the Chicago, IL, metropolitan area. Black dots represent sites where no ticks were detected. Dark red represents areas with the highest developed land cover, while green represents forest cover.

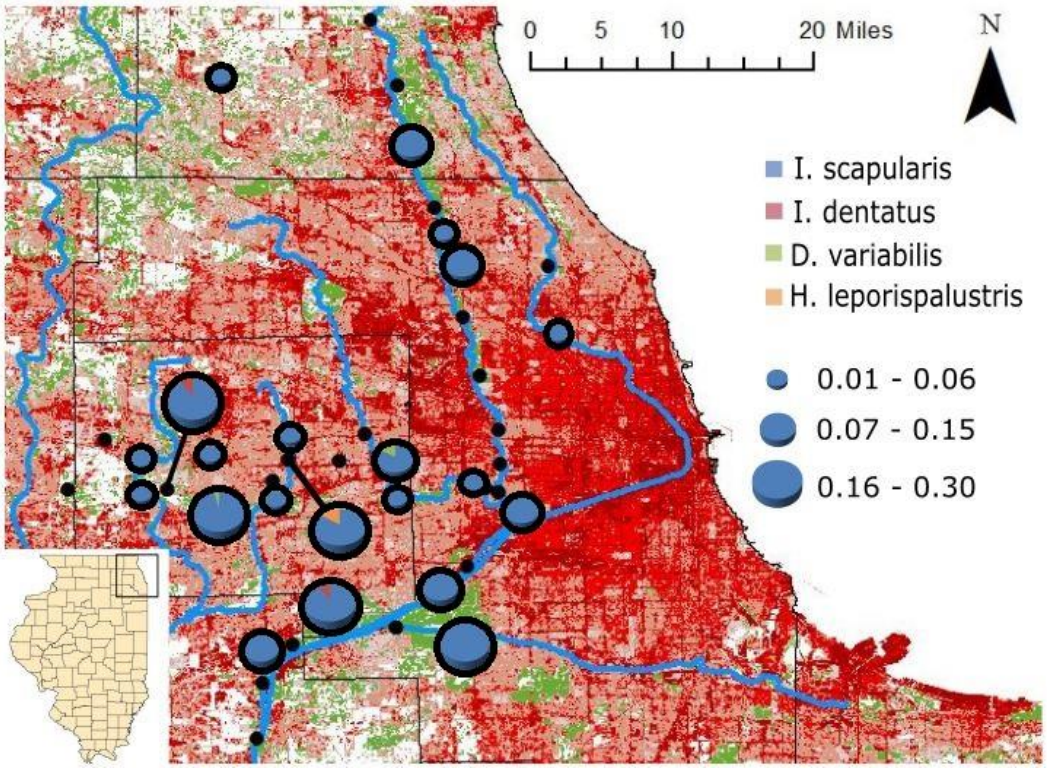

Figure 1b. Abundance of adult *Ixodes scapularis* and other tick species across the Chicago, IL, metropolitan area. Black dots represent sites where no ticks were detected. Dark red represents areas with the highest developed land cover, while green represents forest cover.

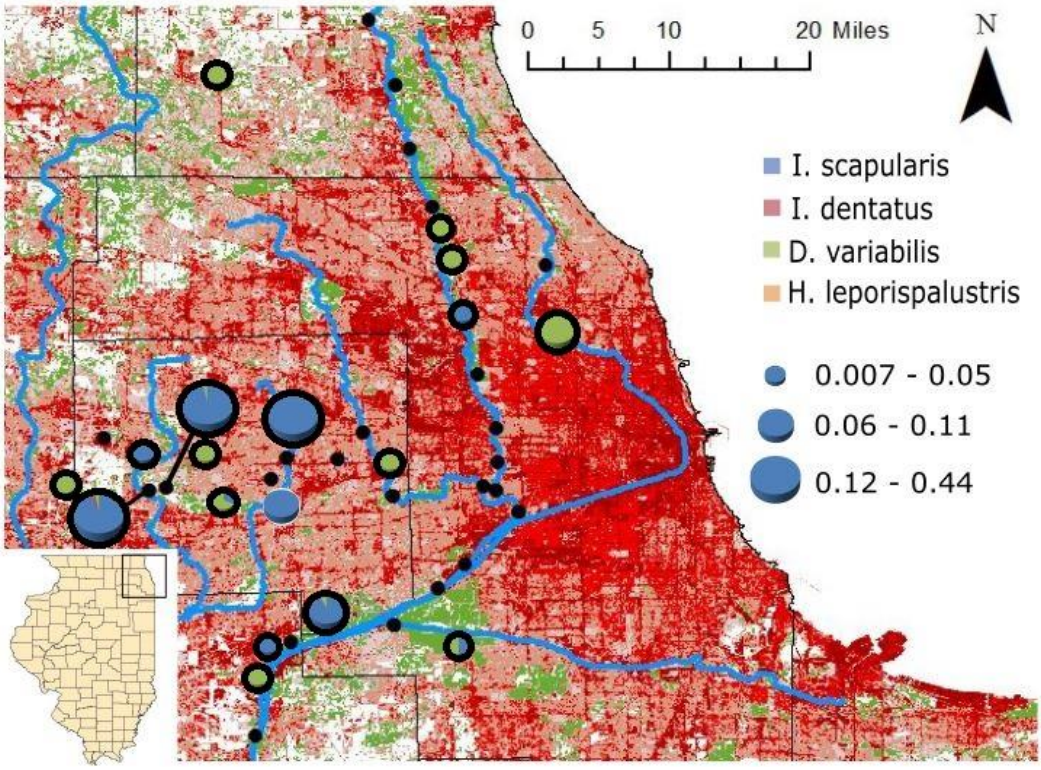

Figure 2a. Diversity and prevalence of pathogens in *Ixodes scapularis* nymphs across the Chicago, IL, metropolitan area. Black dots represent sites where no pathogens were detected. Dark red represents areas with the highest developed land cover, while green represents forest cover.

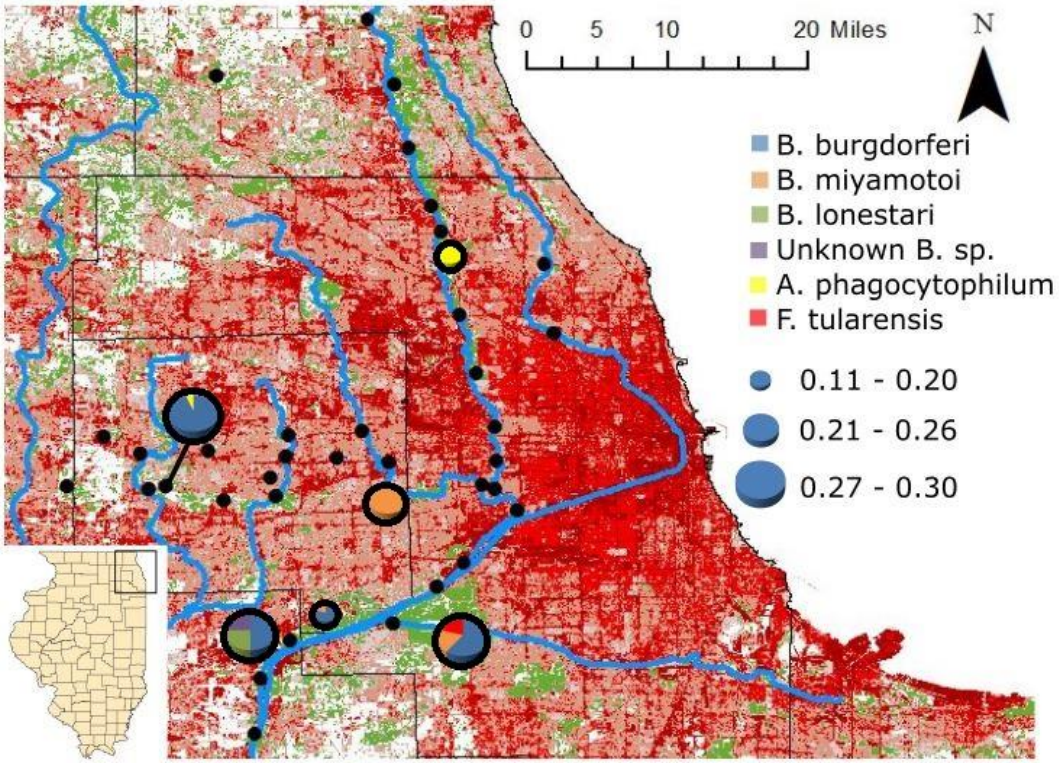

Figure 2b. Diversity and prevalence of pathogens in *Ixodes scapularis* adults across the Chicago, IL, metropolitan area. Black dots represent sites where no pathogens were detected. Dark red represents areas with the highest developed land cover, while green represents forest cover.

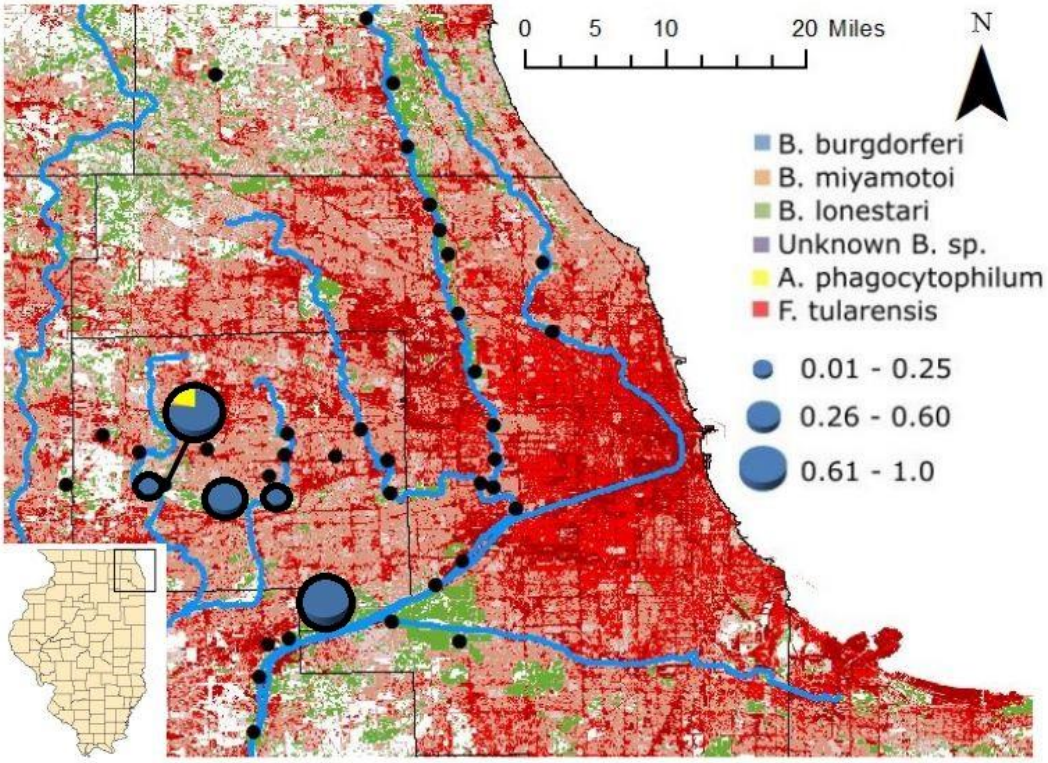

Table 1. Tick sampling effort per year. Observers were researchers from the University of Illinois Urbana-Champaign (UIUC) and DuPage County Forest Preserve District (DCFP).

Year	Sample dates	Observer	Life stage targeted	Number of sites	Number of locations	Number of visits	Avg. drag time per visit (min)
2011	13-14 July	UIUC	Nymphs	17	20	1	60
2012	27 March-11 May	DCFP	Adults	4	15	1	72
2012	26 May-12 July	UIUC	Nymphs	33	35	1	60
2013	20 June-11 July	DCFP	Nymphs	4	4	1-3	50
2013	8 Oct-19 Nov	DCFP	Adults	4	4	1-2	62.5
2013	21 May-2 July	UIUC	Nymphs	31	25	2	43
2014	20 May-24 June	UIUC	Nymphs	46	74	2	19
2014	9 & 16 July	DCFP	Nymphs	2	2	1	45

Table 2. Land cover (L.C.) and distance to nearest major river.

Site Name	Forest L.C. (%), 1000m buffer	Developed L.C. (%), 1000m buffer	Distance from nearest river(m)
Algonquin Woods	2.13%	66.71%	108.11
Allison Woods	28.13%	52.48%	259.95
Beck Lake	35.57%	33.38%	1025.04
Blackwell	24.43%	48.46%	384.2
Brookfield Woods	3.19%	66.43%	163.56
Canoe Launch	21.26%	24.93%	62.42
Churchill Woods	15.49%	76.85%	99.54
Columbia Woods	9.81%	56.60%	82.87
Dam #1 Woods	35.47%	48.83%	116.37
Danada	18.58%	60.41%	2425.03
Edgebrook Woods	4.92%	64.31%	175.14
Fermilab	30.22%	28.03%	3304.71
Fort Sheridan	2.69%	65.39%	262.4
Fullersburg Woods	22.96%	52.38%	155.63
George F. Nixon Woods	16.22%	51.74%	365.86
Glen Oak	0.51%	96.69%	34.61
Hidden Lake	35.34%	47.08%	46.52
Isle a la Cache	7.14%	42.94%	42.5
Keepataw	17.53%	26.08%	688.61
Lakewood	31.28%	20.28%	5923.09
Lincoln Marsh	8.04%	82.51%	2939.42
Lockport Prairie	17.46%	17.25%	121.23
MacArthur Woods	47.86%	11.51%	867.29
Miller Meadows	1.13%	92.06%	61.81
Ottawa Trail Woods	0.91%	75.07%	397.21
Palos/Sagawu	19.95%	39.79%	175.73
Ryerson Woods	29.15%	44.67%	176.48
Salt Creek Greenway	0.00%	100.00%	28.97
Santa Fe Prairie	0.86%	85.10%	85.56
Schiller's Woods	21.82%	42.73%	537.94
Sommes Woods	10.76%	62.35%	2595.5
St. James Farm	24.23%	43.16%	1459.51
Swallow Cliff Woods South	46.69%	10.86%	1372.83
Thatcher Woods	6.33%	69.36%	512.16
Veteran Woods	7.77%	73.28%	1055.66
Waterfall Glen	37.28%	41.02%	2194.35
West Chicago Prairie	16.42%	74.68%	4022.05
West DuPage Woods	15.90%	78.31%	268.02

Table 2 (cont.)

Willowbrook	2.26%	97.25%	1325.25
York Woods	3.56%	95.03%	666.67
Zoo Woods	5.54%	82.90%	182.49

Table 3. Logistic regression model fit.

Model (response variable, buffer size)	AIC Score	ΔAIC
1) I. scapularis, 500m	58.137	4.491
2) I. scapularis, 1000m	53.646	0.00
3) I. scapularis, 1500m	56.145	2.499
4) B. burgdorferi, 500m	38.238	0.00
5) B. burgdorferi, 1000m	41.221	2.983
6) B. burgdorferi, 1500m	43.600	5.362

Table 4. Effects of distance to nearest major river and percent forest cover and percent developed cover within a 1000m site buffer on the presence of *I. scapularis*.

Parameter	Z-score	p-value
Constant	2.333	0.020
Distance	-0.624	0.533
Developed Cover	-2.526	**0.012**
Forest Cover	-2.080	**0.038**

Table 5. Effects of distance to nearest major river and percent forest cover and percent developed cover within a 1000m site buffer on the presence of *B. burgdorferi*.

Parameter	Z-score	p-value
Constant	2.007	0.045
Distance	-0.073	0.942
Developed Cover	-1.377	0.169
Forest Cover	-2.100	**0.036**

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