

Original Contribution

## Invasion of the Lyme Disease Vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* Endemicity

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**Abstract:** Lyme disease risk is increasing in the United States due in part to the spread of blacklegged ticks *Ixodes scapularis*, the principal vector of the spirochetal pathogen *Borrelia burgdorferi*. A 5-year study was undertaken to investigate hypothesized coinvasion of *I. scapularis* and *B. burgdorferi* in Lower Michigan. We tracked the spatial and temporal dynamics of the tick and spirochete using mammal, bird, and vegetation drag sampling at eight field sites along coastal and inland transects originating in a zone of recent *I. scapularis* establishment. We document northward invasion of these ticks along Michigan's west coast during the study period; this pattern was most evident in ticks removed from rodents. *B. burgdorferi* infection prevalences in *I. scapularis* sampled from vegetation in the invasion zone were 9.3% and 36.6% in nymphs and adults, respectively, with the majority of infection (95.1%) found at the most endemic site. There was no evidence of *I. scapularis* invasion along the inland transect; however, low-prevalence *B. burgdorferi* infection was detected in other tick species and in wildlife at inland sites, and at northern coastal sites in years before the arrival of *I. scapularis*. These infections suggest that cryptic *B. burgdorferi* transmission by other vector-competent tick species is occurring in the absence of *I. scapularis*. Other *Borrelia* spirochetes, including those that group with *B. miyamotoi* and *B. andersonii*, were present at a low prevalence within invading ticks and local wildlife. Reports of Lyme disease have increased significantly in the invasion zone in recent years. This rapid blacklegged tick invasion—measurable within 5 years—in combination with cryptic pathogen maintenance suggests a complex ecology of Lyme disease emergence in which wildlife sentinels can provide an early warning of disease emergence.

**Keywords:** *Borrelia burgdorferi*, *Ixodes scapularis*, blacklegged tick, *Peromyscus leucopus*, invasion, Lyme disease

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## INTRODUCTION

Lyme disease accounts for more than 90% of all reported vector-borne disease in the United States with more than 20,000 cases reported annually; its current invasive spread

from endemic foci constitutes a major public health concern (Bacon et al., 2008). In the United States, Lyme disease is caused by the bacterium *Borrelia burgdorferi* and the blacklegged tick *Ixodes scapularis* serves as the vector for most cases. Patterns of human disease mirror the geographic distribution of *B. burgdorferi*-infected *I. scapularis*, which is characterized by high-density endemic foci in the Northeast and Upper Midwestern United States. Increasing incidence is associated with both biological and nonbiological factors, including increases in abundance and range expansions of wildlife, human encroachment into tick habitat, creation of peridomestic habitats that attract wildlife hosts of ticks and pathogens, and enhanced surveillance and awareness among medical professionals. Blacklegged ticks were likely widespread before the most recent glaciation event, during which relict populations remained in refuges from which they and their vertebrate hosts later expanded (Steere et al., 2004). During the past 50 years, white-tailed deer (*Odocoileus virginianus*) populations have undergone explosive growth due to reversion of agricultural lands to forest and restrictions on hunting. Increased deer abundance has facilitated the recent blacklegged tick expansion and Lyme disease emergence throughout the Northeast and Midwest. *I. scapularis* continues to spread from both the Northeastern and Midwestern foci (Steere et al., 2004), but the mechanisms for this spread are not established. *I. scapularis* serves as the vector for multiple zoonotic pathogens, including the agents of Lyme disease, human anaplasmosis, babesiosis, and Powassan encephalitis; therefore, its expansion constitutes increased risk of multiple diseases.

In 2002, a new population of *I. scapularis* was detected in the southwestern corner of Michigan's Lower Peninsula (Foster, 2004). This represented a significant change from the State's previous reported distribution (Walker et al., 1998), which was characterized by a probable lack of established *I. scapularis* throughout the Lower Peninsula. Established populations in the state were recognized at that time only in Menominee County of the Upper Peninsula, adjacent to endemic foci in Wisconsin (Walker et al., 1998). Nevertheless, a Midwestern model of habitat suitability (Guerra et al., 2002) predicted that sandy oak upland forests in Michigan provide highly suitable habitat for *I. scapularis*, and *I. scapularis* indeed were discovered in such areas (Foster, 2004). Suitable habitat types are widespread throughout Lower Michigan, so further invasion of *I. scapularis* is thus predicted.

## Tick Versus Pathogen Invasion?

*B. burgdorferi* and *A. phagocytophilum* have been found within Michigan's recently invaded tick populations (Hamer et al., 2007). The early detection of these low-density infected tick populations afforded us the rare opportunity to test, in real time, three nonexclusive scenarios by which invasion of the Lyme disease vector and/or pathogen into Lower Michigan may be occurring. (1) In the "tick-first" scenario, new uninfected *I. scapularis* populations become established as a result of long distance dispersal of adult ticks by white-tailed deer. This deer-mediated invasion would introduce replete uninfected adult ticks but not *B. burgdorferi* (because deer are incompetent hosts for this pathogen; Telford et al., 1988). In this scenario, we hypothesize that *B. burgdorferi* enters the system later, as result of a slower secondary invasion mediated by infected mammalian or avian hosts. (2) In the "dual-invasion" scenario, mammalian or avian hosts introduce infected *I. scapularis* to new areas so that both *I. scapularis* and *B. burgdorferi* establish concurrently. In this scenario, *B. burgdorferi* should be detectable early in the invasion process, when *I. scapularis* densities are still very low. (3) In the "spirochete-first" scenario, enzootic transmission of the pathogen is maintained by cryptic vectors and reservoir hosts. These vectors are generally wildlife host specialists, which, unlike *I. scapularis*, do not bite humans (Telford and Spielman, 1989a, b), and thus these transmission cycles have no implications for human or canine disease risk. Invasion of *I. scapularis* (infected or uninfected) into zones of cryptic pathogen maintenance will, however, create opportunities for bridging the pathogen to humans and canines.

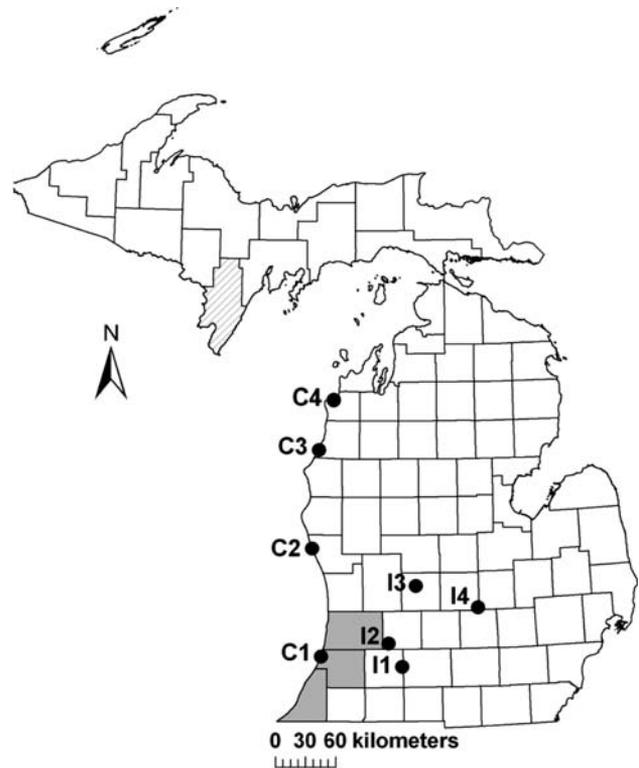
We initiated comprehensive sampling to investigate the spatial and temporal dynamics of the Lyme disease system in and beyond the zone of hypothesized *I. scapularis* invasion in Lower Michigan. To address invasion, we studied areas not only where all three parasitic life stages of *I. scapularis* were endemic (presence of all three stages and/or at least six individuals of a single stage constitute the Centers for Disease Control and Prevention (CDC) definition of an "established" population; Dennis et al., 1998), but also in areas beyond its detected distribution. The objectives of this study were to: (1) test whether the detected distribution of *I. scapularis* changed during the period 2004–2008; and (2) assess the pattern of occurrence of *B. burgdorferi* in relation to that of *I. scapularis*. To address the tick-first hypothesis, each field site was sampled

regularly for the presence of *I. scapularis* to detect new colonization and/or trends in tick abundance at each site. To address the dual-invasion and spirochete-first hypotheses, a diverse community of hosts and their attached ticks were assayed to assess the relationship between *I. scapularis* presence and *B. burgdorferi* infection. Thus, we include results from a diverse assemblage of ticks, including those with low or no vectorial capacity and no associated zoonotic risk (i.e., tick species that are physiologically incompetent for spirochete transmission and/or species that do not regularly feed on human hosts). Although unimportant for transmission, these incompetent ticks can serve as bio-indicators of *B. burgdorferi* presence in an area if they contain an infectious blood meal or transtadially passed spirochetes from a previous meal.

## METHODS

### Site Selection and Sampling Regime

From 2004–2008, we assessed on- and off-host ticks, and wildlife, along two sampling transects established at a spatial scale that extended beyond the known limit of the Lower Michigan *I. scapularis* population (Fig. 1). Both transects originated in the southwestern Michigan zone where *I. scapularis* was first detected in 2002 (Foster, 2004). From this origin, the coastal transect extended north along Lake Michigan (sites labeled C1–C4; 81–145 km between sites: Van Buren State Park, Van Buren Co.; Duck Lake State Park, Muskegon Co.; Orchard Beach State Park, Manistee Co.; Sleeping Bear Dunes National Lakeshore, Benzie Co.; respectively). The inland transect extended northeastward (sites labeled I1–I4; 33–76 km between sites: Fort Custer State Recreation Area, Kalamazoo Co.; Lux Arbor Reserve of Kellogg Biological Station; Barry Co.; Ionia State Recreation Area, Ionia Co.; and Rose Lake Wildlife Research Area, Clinton Co.; respectively). Both transects traversed areas where established *I. scapularis* had not been previously detected (Walker et al., 1998; Foster, 2004), or were uninvestigated. Sampling was conducted in oak-dominated, closed-canopy deciduous forest when available, because this habitat type is positively associated with *I. scapularis* presence in the endemic Midwest (Guerra et al., 2002). Otherwise, sampling was conducted in the dominant forest type at the site. All data presented are for May and June, when *I. scapularis* larvae, nymphs, and adults are simultaneously active. Each site was sampled in



**Figure 1.** Locations of study sites in Lower Michigan, 2004–2008. The four sites along Michigan’s west coast comprise the coastal transect (from south to north, C1–C4), and the four inland sites comprise the inland transect (from southwest to northeast, I1–I4). Shading in the Lower Peninsula represents the three-county region where *I. scapularis* were detected on small mammals in 2002–2003 (Foster 2004a). The cross-hatched county in the Upper Peninsula is Menominee County, Michigan’s longstanding endemic focus of *I. scapularis* and *B. burgdorferi*.

these months in all 5 years of the study, except for site C4 (2007 and 2008 only) and site I1 (all years except 2006).

### Mammal Trapping

At each field site, small mammals were trapped along 4 to 6 transects of 25 Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with sunflower seed. Medium-sized mammals were trapped using 12–16, 32- × 10- × 12-inch live traps (Tomahawk Live Traps, Tomahawk, WI) and wooden box traps baited with peanut butter, jelly, and cat food on tortillas. Small mammals were anesthetized using Isoflurane (IsoFlo, Abbot Laboratories, Abbott Park, IL); medium-sized mammals were anesthetized using ketamine hydrochloride (Ketaset; Fort Dodge, Overland Park, KS) and xylazine hydrochloride (Rompun; Bayer Health Care, Kansas City, KS) followed by reversal with administration of yohimbine

hydrochloride (Antagonil; Wildlife Laboratories, Fort Collins, CO). Animals were identified to species and sex by inspection. Each animal was examined for ticks, biopsied in both ears using a 2-mm (small mammals) or 4-mm (medium mammals) biopsy punch (Miltex Instruments, York, PA), and ear-tagged (National Band and Tag, Newport, KY). Ticks and ear biopsies were stored separately in 70% ethanol. Additional ear biopsies were obtained from animals recaptured after an interval of 2 weeks or more; recaptures at a shorter interval were simply rechecked for ticks. All animals were released at the site of capture. Small mammal trapping success rate was discounted by the number of empty, tripped traps as follows: number mammal captures/(number traps set - 0.5\*no. tripped traps); this expression assumes that on average tripped traps were open for half the night. Wildlife procedures were approved through Michigan State University's Institutional Animal Use and Care Committee permit #02-07-13-000.

### Bird Mist Netting

At each site, six 12-m mist nets (Avinet, Dryden, NY) were used to capture birds in the same areas where mammals were being trapped. Nets were run from 0600–1200 h on fair weather days and checked every 45 min. Birds were weighed, identified to species and sex, measured, searched for ticks, and leg-banded with federally issued bands before release. Mist netting was performed under permit #02640 from the federal Bird Banding Laboratory.

### Questing Tick Sampling

Each site was sampled for questing ticks by dragging a 1-m<sup>2</sup> white corduroy cloth (Falco and Fish, 1992) over the forest floor along the same six transects that were used for mammal trapping. Drag sampling was performed on rain-free days in the late morning or late afternoon to avoid the hottest and least humid times of day (Schulze et al., 2001; Diuk-Wasser et al., 2006). At least 1,000 m<sup>2</sup> of vegetation were sampled per visit. The cloth was inspected every 20 m, and attached ticks were stored in 70% ethanol. Drag sampling was not performed on excessively hot or wet days.

### *Borrelia burgdorferi* Detection

All ticks were identified to species and stage (Keirans and Clifford, 1978; Sonenshine, 1979; Durden and Keirans, 1996). Total DNA from ticks and ear biopsies was extracted

using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol with the following modifications. Ticks were first bisected using a sterile scalpel or were pulverized in liquid nitrogen, followed by an overnight incubation in lysis buffer. DNA was eluted using 50 µl elution solution warmed to 70°C. Ear biopsies (one per animal), adult and nymphal ticks were extracted individually, and conspecific larvae from the same individual animal or drag transect were pooled for extraction. Given our specific questions, we implemented a protocol for subsampling of ticks to assay for infection in the occasional cases of heavily parasitized hosts. In cases where more than three adult or nymphal ticks of the same species, life stage, and sex were removed from an individual host, three were randomly selected for testing (i.e., the maximum number of ticks tested from a host would be, for each tick species present, six adults (three female and three male), three nymphs, and a larval pool). By including a subset of ticks from many hosts, rather than all ticks from a smaller number of hosts (given limited resources), this sampling strategy allowed for improved coverage of the host population and also reduced statistical concerns of nonindependence that would arise if very large numbers of ticks were collected from a small number of highly infested hosts. *B. burgdorferi* strain B31-infected nymphal *I. scapularis* acquired from the CDC and water served as the positive and negative extraction controls, respectively.

*B. burgdorferi* was detected using: (1) a nested polymerase chain reaction (PCR) for the 16S–23S rRNA intergenic spacer region (IGS) of *Borrelia* spp. (Bunikis et al., 2004) followed by visualization with gel electrophoresis, or (2) a quantitative PCR (qPCR) of a region of the 16S rRNA of *B. burgdorferi* (Tsao et al., 2004). PCR enzyme kits were used throughout (nested PCR: PCR Supermix, Invitrogen, Carlsbad, CA and FailSafe PCR System, Epicentre, Madison, WI; qPCR: Universal PCR Master Mix, Applied Biosystems, Foster City, CA). In addition to the positive and negative DNA extraction controls for each batch of samples extracted, DNA from *B. burgdorferi* strain B31-infected ticks from laboratory colonies at the CDC and water served as positive and negative PCR controls. In qPCR, a six-point standard dilution series of DNA extracted from cultured spirochetes ( $10^4$ – $10^{-1}$  organisms per 3 µl reaction volume) served as positive controls (*B. burgdorferi* strain C336, rRNA spacer type (RST) II strain). Reactions for qPCR were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Preliminary experiments showed that

both tests were able to detect positive samples containing a minimum  $10^0$ – $10^{-1}$  organisms.

### Nucleotide Sequencing

A subset of *B. burgdorferi*-positive ticks and ear biopsies were DNA sequenced to confirm pathogen identity. Samples included: (1) a random subset of positive samples from sites where *I. scapularis* is common; (2) a majority of test-positive samples from sites with an apparent absence of *I. scapularis*; and (3) any IGS amplicons of approximately 500 bp, characteristic of the relapsing-fever spirochete *B. miyamotoi* (cf. the 987 bp fragment characteristic of *B. burgdorferi*). When a sample was determined to be positive using qPCR, IGS PCR was then performed to generate the template for sequencing. The IGS product was purified (Qiagen PCR Purification Kit; Qiagen, Valencia, CA) and sequences were determined in both directions using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were identified as *B. burgdorferi* or *B. miyamotoi* based on comparisons to published sequences using the basic local alignment search tool in GenBank (Altschul et al., 1990). *B. burgdorferi* strains were assigned to IGS ribosomal spacer type (RST; Liveris et al., 1995) by alignment with the prototypical strains published in Bunikis et al. (2004) using the program MEGA (Tamura et al., 2007).

### Statistics

Logistic regression was used to assess trends in wildlife infestation and tick infection during the 5-year sampling period. Fisher's exact test was used to assess differences in infestation among sites. Linear regression was used to assess trends in nymphal densities within sites over time. Within-year comparisons between the coastal and inland transects were made by calculating the z-ratio and associated two-tail probabilities for the difference between two independent proportions. Minimum infection prevalence (MIP; i.e., assuming only one positive larva per pool) was used for tests done on pooled larvae. Statistics were performed using Statistix 8 (Analytical Software, Tallahassee, FL).

## RESULTS

### Wildlife Captures

We achieved a total of 1,667 mammal captures, comprised of 1,472 small and 195 medium mammal captures. Small

mammals were captured in a total of 7,998 adjusted trap nights (18.4% overall capture rate). White-footed mouse (*Peromyscus leucopus*) was the most commonly caught species (84.9% of all small mammal captures); their capture rates did not differ between coastal and inland transect sites ( $P = 0.95$ ). Medium mammals were captured in a total of 777 adjusted trap nights (25.1% overall capture rate), with similar rates along both transects ( $P = 0.93$ ). Raccoon (*Procyon lotor*) was the most abundant species (65.6% of all medium mammal captures). Despite escaped animals and the release of lethargic shrews (*Blarina brevicauda*), 99.8% of captured mammals were processed, 82.5% of which were first captures, and 6.5% were recaptures after a minimum 2-week interval; these animals were processed in full and used for computing the infestation and infection prevalences reported below. The remaining 11% represent white-footed mice recaptured within 2 weeks. These latter mice are not considered further in infestation analyses; however, ticks removed from these animals are considered in infection analyses. In total, 14 mammal species were parasitized by 8 species of ticks (Table 1).

A total of 747 bird captures, comprised of 55 species, occurred in 2,180 net hours for an average of 34.3 birds per 100 net hours. Due to escaped birds, 99.2% were processed, of which 91.6% represent first captures and 6.7% were bird recaptured after a minimum 2-week interval; these birds were processed in full and used in computing infestation and infection prevalences below. The remaining 1.6% represents birds recaptured within 2 weeks—none harbored ticks. Birds were parasitized by three species of ticks (Table 1; Supplement 1).

### *I. scapularis* on Wildlife

Along the inland transect, *I. scapularis* were rarely found on wildlife, with only 3 of 574 white-footed mice parasitized. Two of these were trapped at site I3 in 2007 (3% infestation), with one harboring a single larva and nymph, and one harboring four larvae. The other mouse was trapped at site I1 in 2008 (3.9% infestation) and harbored a single nymph. None of the 73 eastern chipmunks, 133 individuals of other mammal species, or 504 birds inspected on this inland transect harbored *I. scapularis*.

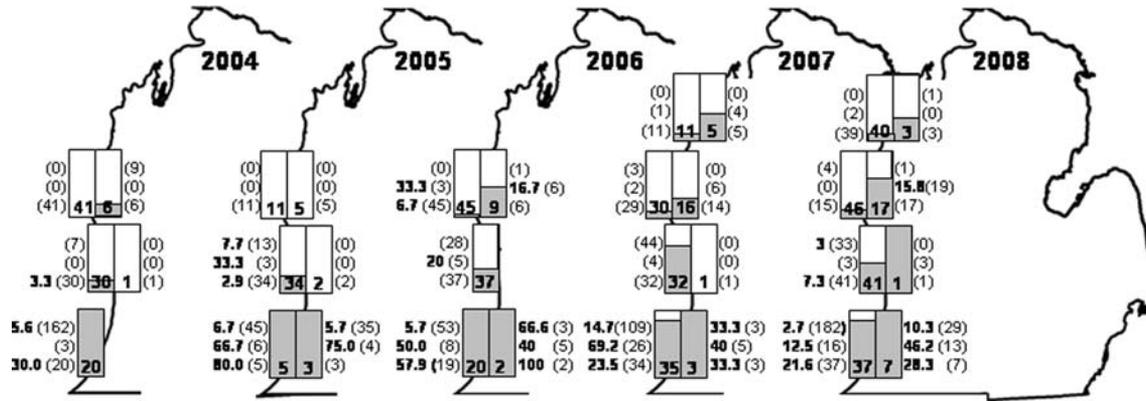
In contrast, a steep gradient of *I. scapularis* infestation of wildlife was evident along the coastal transect. In all years the level of white-footed mouse infestation was highest at the southernmost C1 site (75–100% with no significant difference among years;  $P = 0.08$ ), and larval and nymphal

**Table 1.** Tick-Host Associations and Infestation Prevalence on the Inland and Coastal Transects in Lower Michigan, May–June, 2004–2008

Host species	Transect	No. captures	No. animals infested with L/N/A (% infested with any stage)										
			<i>D. variabilis</i>	<i>I. scapularis</i>	<i>I. texanus</i>	<i>I. cookei</i>	<i>I. dentatus</i>	<i>I. marxi</i>	<i>H. leporispalustris</i>	<i>A. americanum</i>			
Northern short-tailed shrew	Inland	10											
<i>Blarina brevicauda</i>	Coastal	3											
Virginia opossum	Inland	22	0/0/15 (68.2)			1/1/1 (9.1)							
<i>Didelphis virginiana</i>	Coastal	13	0/0/6 (46.2)	6/8/2 (61.5)		0/3/0 (23.1)							
Southern flying squirrel	Inland	1											
<i>Glaucomys volans</i>	Coastal	13	1/0/2 (23.1)	0/2/0 (15.4)	1/0/0 (7.7)			0/0/1 (7.7)					
Striped skunk													
<i>Mephitis mephitis</i>	Coastal	2		0/1/0 (50)		1/1/1 (100)							
Meadow vole													
<i>Microtus pennsylvanicus</i>	Coastal	1		1/0/0 (100)									
Long-tailed weasel													
<i>Mustela frenata</i>	Inland	2	1/0/0 (50)			1/1/0 (50)							
White-footed mouse	Inland	665	293/58/0 (46.2)	2/2/0 (0.5)									
<i>Peromyscus leucopus</i>	Coastal	570	106/42/0 (20.3)	175/53/0 (33.5)	1/1/0 (0.4)								
Raccoon	Inland	64	1/4/37 (59.4)			6/26/32 (68.8)	1/6/1 (10.9)						
<i>Procyon lotor</i>	Coastal	62	2/1/39 (62.9)	3/11/2 (21.0)	10/34/34 (72.6)	0/5/2 (9.7)	0/1/0 (1.6)	0/0/1 (1.6)				0/0/1 (1.6)	
Eastern gray squirrel													
<i>Sciurus carolinensis</i>	Coastal	5	1/0/1 (20)	2/4/0 (80)									
Fox squirrel													
<i>Sciurus niger</i>	Coastal	1		1/1/0 (100)									
Eastern cottontail	Inland	4	0/0/1 (25)					0/1/4 (100)				0/0/1 (25)	
<i>Sylvalagus floridanus</i>	Coastal	1						0/0/1 (100)				0/1/1 (100)	
Eastern chipmunk	Inland	74	3/2/0 (6.8)										
<i>Tamias striatus</i>	Coastal	84	1/0/0 (1.2)	16/36/0 (46.4)	1/0/0 (1.2)								
Red squirrel	Inland	16	0/1/0 (6.3)		0/0/1 (6.3)	0/1/0 (6.3)			0/1/1 (12.5)				
<i>Tamiasciurus hudsonicus</i>	Coastal	2		1/0/0 (50)	0/0/1 (50)								
Meadow jumping mouse	Inland	12	6/1/0 (50)					4/0/0 (33.3)					
<i>Zapus hudsonius</i>	Coastal	16		1/2/0 (12.5)				0/0/1 (6.3)				1/5/6 (1.2)	
Birds <sup>a</sup>	Inland	504						57/29/0 (12.9)				2/2/0 (1.3)	
All species	Coastal	237		5/14/0 (5.9)				4/4/2 (3.8)					

<sup>a</sup>Species-specific bird captures and tick associations are presented in Supplement 1.

N nymph, A adult, L larva.



**Figure 2.** Infestation of white-footed mouse (left rectangle) and eastern chipmunk (right rectangle) with *I. scapularis* at the four sites of the coastal transect, May and June, 2004–2008. Level of shading indicates the proportion of animals infested, with the number of animals screened for ticks inside the symbol. Adjacent to each symbol, *B. burgdorferi* infection prevalence (%) in (1) *I. scapularis* larvae

burdens averaged 4.7 and 0.6 per mouse, and 5.6 and 2.3 per infested mouse (maxima of 37 and 11, respectively). Infestation at the more northern sites was much lower but increased progressively during the study (Fig. 2). At site C2, *I. scapularis*-infested mice were trapped in all 5 years, with infestation increasing significantly over time ( $P < 0.001$ ). At site C3, no *I. scapularis*-infested mice were observed until the third summer after which infestation increased ( $P = 0.76$ ). Site C4 harbored a low proportion of *I. scapularis*-infested mice in both 2007 and 2008 (9% and 10%, respectively); these were the only 2 years when May–June sampling was conducted at this site (in July 2005 none of 21 mice trapped at C4 harbored *I. scapularis*, whereas 60% of 110, 13% of 54, and 0% of 16 mice were infested at sites C1, C2, and C3 respectively).

Overall, 48.2% of 81 eastern chipmunks harbored *I. scapularis* along coastal sites. As with the mice, infestation was highest at site C1, with all chipmunks there ( $n = 15$ ) infested in all years. Fewer chipmunks were infested at the three more northern sites (Fig. 2;  $P < 0.0001$ ); however, infestation levels were increasing over time ( $P = 0.01$  for sites C2 and C3 pooled). *I. scapularis* burdens on chipmunks were greatest at site C1; larval burdens there were similar to those of mice (average 4.7 per chipmunk and 5.8 per larval-infested chipmunk, maximum of 20), whereas nymphal burdens were much higher (average 7.2 per chipmunk, and 7.7 per nymphal-infested chipmunk, maximum of 36). Of 117 other mammals caught on the coastal transect, 28.2% comprising ten species harbored *I. scapularis* (Table 1); most were from C1

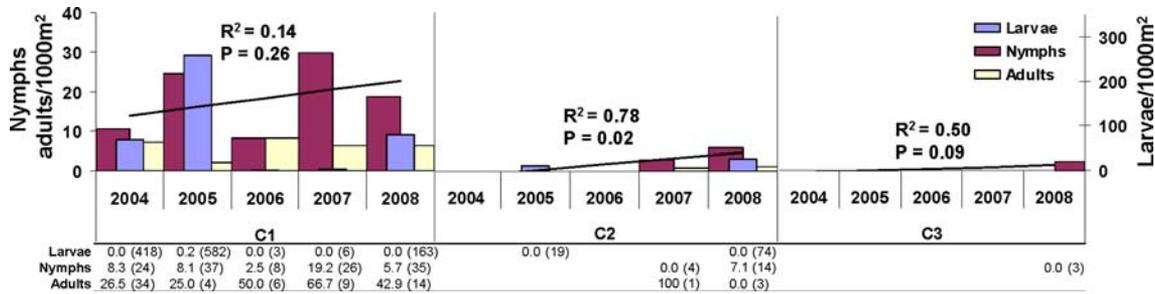
(minimum infection prevalence), (2) nymphs removed from hosts, and (3) host ear biopsies are listed from top to bottom, each followed by sample size in parenthesis. At site C4, no traps were set in 2004–2006. At site C1 in 2004 and C2 in 2006, no chipmunks were captured despite traps being set. Information on the inland transect sites, where infestation and infection rates were close to zero, appears in the text.

(54.5%) or C2 (24.2%). Of 237 bird captures, *I. scapularis* was found on 14, of which 13 were from C1 and one was from C2. These 14 birds comprised five species: American robin, chipping sparrow, Eastern towhee, indigo bunting, and Northern cardinal (Supplement 1).

### Questing Ticks

A total of 58,459 m<sup>2</sup> of drag sampling was conducted, with 26,800 and 31,650 m<sup>2</sup> along the inland and coastal transects, respectively. A total of 1,984 ticks were collected of which 87% were *I. scapularis*. Four other tick species were collected on drag cloth: *Dermacentor variabilis* ( $n = 252$ ), *Amblyomma americanum* ( $n = 4$ ), *Haemaphysalis leporispalustris* ( $n = 2$ ), and *I. dentatus* ( $n = 5$ ).

All but one *I. scapularis* came from coastal sites. Along the inland transect, a single larva was dragged (at I3 in 2007). Conversely, along the coastal transect, 86 adults, 246 nymphs, and 1,388 larvae were collected, of which 95.3%, 91.5%, and 93.3% respectively were from site C1. A significant gradient of *I. scapularis* density was detected, with the greatest abundance at site C1, where all life stages were present in all five summers of sampling (peak May/June densities were 8.2 adults, 29.7 nymphs, and 90.8 larvae per 1000 m<sup>2</sup>) and nymphal abundance did not change significantly during the 5 years ( $R^2 = 0.14$ ;  $P = 0.26$ ). Many fewer, yet increasing, numbers of *I. scapularis* occurred at the two sites to the north (Fig. 3): at site C2, no *I. scapularis* were dragged at the start of the study whereas all stages were present by the end with a significant increase in



**Figure 3.** Average densities and *B. burgdorferi* infection prevalences of drag-sampled *I. scapularis* at the three sites on the coastal transect in May and June, 2004–2008. No *I. scapularis* were dragged at site C4. Regression lines and coefficients for nymphal densities are

nymphal density ( $R^2 = 0.78$ ;  $P = 0.02$ ). At site C3, an increase in nymphal density occurred ( $R^2 = 0.05$ ,  $P = 0.09$ ), and this was the only life stage found on drag cloths. No questing *I. scapularis* were collected at site C4.

### *B. burgdorferi* Infection in Drag-sampled Ticks

Along the coastal transect, 36.6%, 9.3%, and a minimum of 0.1% of adult, nymphal, and larval *I. scapularis* respectively were infected with *B. burgdorferi*, with the majority of infected ticks (95.1%) collected at site C1 (Fig. 3). There was no significant change in infection prevalence among years at site C1 ( $P = 0.38$ – $0.71$  for the three age classes); too few positive ticks were collected at other coastal sites to test for equivalent trends. Along the inland transect, the single *I. scapularis* larva that was dragged at site I3 in 2007 tested positive for *B. burgdorferi* (Table 2). Of all *D. variabilis* collected on drag cloth, *B. burgdorferi* was found in 8 of 147 adults, 0 of 4 nymphs, and a minimum of 1 of 59 larvae processed in 15 pools, again with no apparent temporal trends. All infected drag-sampled *D. variabilis* came from site C1, with the exception of two infected adults from sites I1 and I2. *B. burgdorferi* detection in other drag-sampled tick species across the years was as follows: 0 of 4 *A. americanum*, 0 of 1 *A. maculatum*, 0 of 1 *I. cookei*, 2 of 2 *H. leporispalustris* (both nymphs from site C1), and 0 of 3 *I. dentatus*.

### Host Infection with *B. burgdorferi*

Of the 1,056 white-footed mouse ear biopsies that were collected from 2004–2008, only 2.1% of 576 tested positive from inland sites (I1: 0/71; I2: 9/213; I3: 3/169; I4: 0/121, with no apparent trend over time), whereas 11.9% of 480 coastal mice tested positive ( $P < 0.0002$ ). Infection prevalence was high in all years at site C1 (21.6–80%), and low and inconsistent at sites further north (Fig. 2). A total of 148 chipmunk biopsies were assayed for infection, of which 4.2% of 72 were positive along the inland transect (I1: 0/9; I2: 0/20; I3: 0/4; I4: 3/39), and 6.6% of 76 (all at site C1) were positive along the coastal transect (Fig. 2; no difference between transects,  $P = 0.72$ ). A total of 240 ear biopsies from alternative mammal species were assayed for infection: 6.5% of 124 and 4.3% of 116 were positive along the inland and coastal transects, respectively (no difference between transects,  $P = 0.57$ ). Positive alternative mammals comprised four species (raccoon, red squirrel (*Tamiasciurus hudsonicus*), meadow jumping mouse (*Zapus hudsonianus*), and Virginia opossum (*Didelphus virginiana*) at two inland sites (I3 and I4) and three species (eastern gray squirrel (*Sciurus carolinensis*), raccoon, and meadow jumping mouse) at two coastal sites (C1 and C3).

shown. Infection prevalence for each life stage (minimum infection prevalence for larvae) is expressed as the percent positive, with the total number tested in parenthesis below each year. C = coastal transect site.

alence was high in all years at site C1 (21.6–80%), and low and inconsistent at sites further north (Fig. 2). A total of 148 chipmunk biopsies were assayed for infection, of which 4.2% of 72 were positive along the inland transect (I1: 0/9; I2: 0/20; I3: 0/4; I4: 3/39), and 6.6% of 76 (all at site C1) were positive along the coastal transect (Fig. 2; no difference between transects,  $P = 0.72$ ). A total of 240 ear biopsies from alternative mammal species were assayed for infection: 6.5% of 124 and 4.3% of 116 were positive along the inland and coastal transects, respectively (no difference between transects,  $P = 0.57$ ). Positive alternative mammals comprised four species (raccoon, red squirrel (*Tamiasciurus hudsonicus*), meadow jumping mouse (*Zapus hudsonianus*), and Virginia opossum (*Didelphus virginiana*) at two inland sites (I3 and I4) and three species (eastern gray squirrel (*Sciurus carolinensis*), raccoon, and meadow jumping mouse) at two coastal sites (C1 and C3).

### *B. burgdorferi* infection in *I. scapularis* removed from hosts

Along the inland transect, all five larvae removed from two mice at site I3 in 2007 were negative, whereas the single nymph from the same site and year was positive (Table 2), and the single nymph from site I1 in 2008 was negative. Along the coastal transect, infection in *I. scapularis* removed from mice and chipmunks was highest at site C1 and decreased at sites to the north in all years, with no infection in the ticks from site C4 (Fig. 2). Alternative mammalian hosts harboring *I. scapularis* were found at all coastal sites, but *B. burgdorferi* infection in these ticks was found only at sites C1 and C2 (Supplement 2). Birds harboring *I. scapularis* were found only at sites C1 and C2, and *B. burgdorferi* infection in bird-derived *I. scapularis* occurred only at site C1 (Supplement 2).

**Table 2.** Sequence Confirmation and Accession Numbers of *B. burgdorferi* Infection in Various Sample Types from Coastal and Inland Sites in Lower Michigan, 2004–2008

Site	Year	Sample type	Host species or drag	RST (1, 2, 3)	Acc. No
C1	2004	<i>D. variabilis</i> AF	Drag	2	GQ856591
		Ear biopsy	WFMO	1	GQ856630
		Ear biopsy	WFMO	2	GQ856631
		<i>I. marxi</i> AF	SFSQ	1	GQ856615
		<i>I. scapularis</i> L	WFMO	2	GQ856598
		<i>I. scapularis</i> N	EAGS	2	GQ856614
	2005	<i>D. variabilis</i> L	Drag	1	GQ856596
		<i>D. variabilis</i> L	WFMO	3	GQ856600
		<i>I. scapularis</i> N	VIOP	2	GQ856594
		<i>I. scapularis</i> N	WFMO	3	GQ856595
		<i>I. texanus</i> L	SFSQ	2	GQ856601
		2006	<i>D. variabilis</i> N	WFMO	2
	<i>I. scapularis</i> N		Drag	2	GQ856592
	<i>I. scapularis</i> N		EACH	1	GQ856612
	<i>I. scapularis</i> N		WFMO	2	GQ856611
	2007	<i>D. variabilis</i> N	WFMO	3	GQ856643
		Ear biopsy	EACH	3	GQ856632
		<i>H. leporispalustris</i> N	Drag	3	GQ856609
		<i>I. scapularis</i> L	NOCA	3	GQ856619
<i>I. scapularis</i> N		AMRO	3	GQ856616	
C2	2005	Ear biopsy	WFMO	2	GQ856620
	2006	<i>I. scapularis</i> L	WFMO	2	GQ856593
	2008	Ear biopsy	WFMO	2	GQ856610
C3	2006	Ear biopsy	WFMO	3	GQ856623
		Ear biopsy	WFMO	3	GQ856622
		Ear biopsy	WFMO	3	GQ856621
I2	2004	<i>D. variabilis</i> L	WFMO	1	GQ856638
	2005	<i>D. variabilis</i> L	WFMO	1	GQ856602
	2006	Ear biopsy	WFMO	2	GQ856628
		Ear biopsy	WFMO	2	GQ856629
		Ear biopsy	WFMO	2	GQ856627
		Ear biopsy	WFMO	2	GQ856626
		Ear biopsy	WFMO	2	GQ856625
		Ear biopsy	WFMO	2	GQ856624
		Ear biopsy	WFMO	2	GQ856623
	2007	<i>D. variabilis</i> L	WFMO	3	GQ856603
I3	2004	<i>D. variabilis</i> L	WFMO	2	GQ856599
		<i>D. variabilis</i> L	WFMO	2	GQ856597
		<i>I. texanus</i> N	COON	3	GQ856640
	2006	Ear biopsy	WFMO	2	GQ856642
		Ear biopsy	MJMO	2	GQ856641
	2007	<i>I. scapularis</i> N	WFMO	2	GQ856606
		<i>I. scapularis</i> L	Drag	2	GU190359
		<i>D. variabilis</i> L	WFMO	2	GQ856608
		<i>D. variabilis</i> L	WFMO	2	GQ856607
		<i>D. variabilis</i> L	WFMO	2	GQ856605
	<i>D. variabilis</i> L	WFMO	2	GQ856604	

**Table 2.** continued

Site	Year	Sample type	Host species or drag	RST (1, 2, 3)	Acc. No
		Ear biopsy	VIOP	2	GQ856636
		Ear biopsy	COON	2	GQ856634
		Ear biopsy	COON	2	GQ856635
		Ear biopsy	WFMO	2	GQ856633
	2008	<i>I. dentatus</i> AF	EACO	2	GQ856618
		<i>I. dentatus</i> AM	EACO	2	GQ856617
I4	2004	<i>I. texanus</i> N	COON	1	GQ856613
	2006	Ear biopsy	EACH	2	GQ856637

RST = 16S–23S rRNA spacer type of *B. burgdorferi*.

I inland transect site, C coastal transect site, AF adult female, AM adult male, N nymph, L larvae, WFMO white-footed mouse, SFSQ southern flying squirrel, EAGS eastern gray squirrel, VIOP Virginia opossum, EACH eastern chipmunk, NOCA Northern cardinal, AMRO American robin, SOSP song sparrow, COON raccoon, MJMO meadow jumping mouse, EACO eastern cottontail.

### ***B. burgdorferi* Infection in Alternative Tick Species**

Assaying samples of the seven species of ticks other than *I. scapularis* removed from mammals and birds revealed relatively high levels of *B. burgdorferi* infection in alternative ticks at sites with sympatric *I. scapularis* (Supplement 2). Additionally, some alternative tick species from inland sites—in the apparent absence of *I. scapularis*—were associated with low-level infection prevalence. Infected *D. variabilis* removed from mice were found at sites I2 and I3, where a total of 7.1% of 98 nymphs (positives from 6 animals) and a minimum of 0.7% of 2006 larvae (positives from 14 animals) collected at these two sites together were infected. This contrasts with site C1, where sympatric *I. scapularis* occur in high density: 17.6% of 74 nymphal (positives from 12 animals) and a minimum of 2.5% of 672 larval (positives from 17 animals) *D. variabilis* removed from white-footed mice were infected.

Of the alternative tick species collected from vertebrates, *I. texanus* was the most abundant and was ubiquitous across our study sites, commonly found parasitizing raccoons (Table 1). Infected attached *I. texanus* were found not only at all coastal transect sites, but also in all three tick life stages from inland sites (2.4–2.6%; Supplement 2). In summary, infected *D. variabilis*, *I. texanus*, *I. cookei*, *I. dentatus*, *I. marxi*, and *H. leporispalustris* were removed from coastal wildlife, and infected *D. variabilis*, *I. texanus*, and *I. dentatus* were removed from inland wildlife (Supplement 2).

### **Nucleotide Sequencing**

Pathogen identity was confirmed by nucleotide sequencing of the 16S–23S rRNA IGS region of a subset of the PCR positive samples reported earlier (i.e., from host ear tissue, *I. scapularis* ticks, and other tick species). We obtained sequence confirmation of 26 samples from coastal sites where *I. scapularis* is abundant and 28 samples from inland sites where *I. scapularis* is absent or at a very low density. Representatives of all three *B. burgdorferi* RST groups were found at sites characterized by both the presence and absence of *I. scapularis* (Table 2).

### **Opportunistic Detection of other *Borrelia* Species**

Using IGS PCR followed by DNA sequencing, we detected spirochetes that group with *B. miyamotoi*, an organism genetically similar to relapsing fever group spirochetes, in ears from 12 white-footed mice at sites C2–C4. Two tick samples (a single nymph and a pool of 20 larval *I. scapularis*) removed from the same eastern chipmunk in 2005 at site C1 also tested positive for *B. miyamotoi*-like spirochetes. Representative *B. miyamotoi*-like sequences from these ears and ticks are deposited in Genbank (accession numbers GQ856588–GQ856589). We also detected spirochetes that group with *B. andersonii*, a Lyme borreliosis group spirochete associated with *I. dentatus* ticks, birds, and rabbits (Marconi et al., 1995), in one *I. dentatus* nymph removed from a song sparrow from site I3 in 2007 (GQ856590) and in ear tissue from one white-footed

mouse from cite C1 in 2006. Because the IGS region of *B. andersonii* was not previously characterized in Genbank, we identified this species based on characterization of the 16S gene (data not shown).

## DISCUSSION

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Lyme disease is emerging in the United States largely due to the spread of the bridging vector, *I. scapularis*. Both in the Northeast and the Upper Midwest, the spread of this tick has been documented at a gross scale, but few have studied this spread in real time (Falco et al., 1995, Schulze et al., 1998, and Cortinas and Kitron, 2006 are exceptions). By monitoring ticks and hosts for 5 years, we assessed the temporal and spatial dynamics of *I. scapularis* populations along coastal and inland transects in western Lower Michigan. Our goal was to investigate three nonmutually exclusive hypotheses explaining how the disease system is emerging across the landscape; we refer to these as the tick-first, dual-invasion, and spirochete-first hypotheses. Our results suggest that there are distinct but overlapping processes that determine *I. scapularis*/*B. burgdorferi* dynamics. In some areas, observations of newly arrived ticks and spirochetes in host/vector communities where *B. burgdorferi* had not been detected support the tick-first and/or dual-invasion hypotheses. Conversely, in other areas, the detection of spirochete-infected wildlife and alternative tick species preceded detection of *I. scapularis*, which is consistent with the spirochete-first hypothesis. We summarize our key findings and consider their support for these hypotheses.

### *I. scapularis* Invasion

At a broad spatial scale, a continuum of endemicity of *I. scapularis* exists in the Midwest, whereby Wisconsin, Minnesota, and a focal area in Michigan's Upper Peninsula harbor long-established tick populations (Jackson and Defoliart, 1970; Drew et al., 1988; Strand et al., 1992). Illinois and Indiana harbor ticks that colonized later (Pinger and Glancy, 1989; Bouseman et al., 1990), and Michigan's Lower Peninsula represents the most recent invasion front (Foster, 2004; Hamer et al., 2007). We postulate that these Michigan ticks represent an expanding focus from recently described populations in northern Indiana (near the Michigan border and Lake Michigan; Pinger et al., 1996). The density of questing nymphs at

coastal site C1, where *I. scapularis* was most common, averaged 16.8/1000 m<sup>2</sup>, which is higher than the average density of 6.6/1000 m<sup>2</sup> in *I. scapularis*-positive sites from throughout the eastern United States (Diuk-Wasser et al., 2006). Densities of nymphs at the other *I. scapularis*-positive sites in our study were much lower.

As in many other states that have witnessed an emergence of Lyme disease during the past four decades, white-tailed deer populations have increased sharply in Michigan's southern Lower Peninsula for the past 50 years, from near extirpation in the 1960s to nearly one million animals presently as a consequence of harvest restrictions and reforestation (MDNR, 2002). Deer densities throughout the invasion zone are clearly above the threshold necessary to support blacklegged tick populations. The reforestation that supported the deer expansion also created an abundance of suitable habitat for other woodland hosts and *I. scapularis*. Thus, the observed tick invasion may be a consequence of a sufficient number of introductions and a large enough propagule size deposited in southwestern Michigan to achieve the density needed to establish and disperse.

The progressive increase in tick density along the coastal sites from south to north among years is suggestive of rapid tick invasion and colonization northward along the coastal dune forests of Lake Michigan. *I. scapularis* could only be detected at the southernmost site at the beginning of the study, whereas all four sites harbored established populations by the end of the study period. Numerous authors have proposed that climate change may facilitate tick invasion of new areas due to a warming of the microclimate experienced by the ticks and shorter periods of freeze (e.g., Githeko et al., 2000; Lindgren and Gustafson, 2001). The prospects for such shifts are debatable (Randolph, 2004), and *I. scapularis* in Lower Michigan is well south of its distributional limits, so we presently have no evidence to suggest climate change as a driver for this invasion.

The disparity in tick distribution between our two transects suggests that there are important ecological differences between coastal and inland sites (Sonenshine et al., 1995), with the former allowing for many more introductions of ticks, and/or higher success in establishment after introduction. Hosts with high tick burdens and large home ranges, such as deer and migratory birds, are critical for long distance dispersal of *I. scapularis*, whereas small mammals with small home ranges may limit invasion by diverting ticks from more vagile hosts (Madhav et al.,

2004). Deer densities were not quantified in our study, but densities of other mammalian and nonmigratory birds during the study period, as indexed by our trap and mist-net success, were similar between coastal and inland sites. Migratory birds tend to concentrate along shorelines (Alerstam, 1978) and fly parallel to the long axis of Lake Michigan (Diehl et al., 2003), which may allow for more drop-offs of ticks from birds at coastal compared with inland sites.

### ***B. burgdorferi* Distribution, Abundance, and Diversity**

Overall infection prevalences in drag-sampled *I. scapularis* in the invasion zone were 8.6% and 36.6% in nymphs and adults, respectively; these infection prevalences are lower than those characteristic of *I. scapularis* in the endemic Northeast (15% in nymphs (Gatewood et al., 2009); 49% in adults (Schulze et al., 2003)). The density of infected nymphs (DIN) is correlated with the incidence of human Lyme disease in a given area (Stafford et al., 1998). Across our study, the DIN at site C1 was 1.5/1000 m<sup>2</sup>, which is similar to DINs in endemic sites of the Northeast (1.3/1000 m<sup>2</sup>) and Midwest (1.0/1000 m<sup>2</sup>; Gatewood et al., 2009). The overall prevalence of infection of white-footed mice at site C1 was 32.2%, which is lower than that reported from endemic foci (for example, 76% by the end of summer in Connecticut; Barbour et al., 2009). In addition to the lower nymphal infection prevalence as an explanation for lower prevalence of mouse infection, the difference may be due in part to sampling prior to the end of the nymphal host-seeking, and therefore transmission, period.

We detected *B. burgdorferi* not only in sites where *I. scapularis* is established or apparently recently invaded, but also within alternative tick species and wildlife hosts occurring at sites in the apparent absence of *I. scapularis*. Cryptic transmission of *B. burgdorferi* sensu lato has been documented in various host and tick species (Anderson et al., 1989; Telford and Spielman, 1989b; Brown and Lane, 1992; Maupin et al., 1994; Oliver et al., 2003) but has not previously been investigated in an area of *I. scapularis* invasion. The requirements for demonstration of a cryptic transmission cycle include the presence and interactions of (1) *B. burgdorferi*, (2) a vector-competent tick species, and (3) reservoir-competent hosts. Although our data confirm the presence of many reservoir competent hosts, and infection with several strains of *B. burgdorferi* in such hosts and in various tick species—all suggestive of cryptic cy-

cles—it remains unclear which tick species are maintaining transmission of *B. burgdorferi* in the absence of *I. scapularis*. We documented infection in both questing and on-host *D. variabilis*, as did Walker et al. (1998) at a highly endemic area in Michigan's Upper Peninsula, yet this tick species is not considered a competent vector (Piesman and Sinsky, 1988). *Ixodes cookei* and *I. marxi* both had a low prevalence of infection in our study, but these infected ticks were removed from animals (southern flying squirrel, raccoon) only at site C1, where *I. scapularis* and *B. burgdorferi* were at relatively high prevalence; infections in these alternative tick species thus may represent spillover from feeding on hosts infected by *I. scapularis*. Furthermore, *I. cookei* is an incompetent vector (Ryder et al., 1992). Two infected tick species removed from wildlife at inland sites included *I. texanus* and *I. dentatus*. Although the vector competency of *I. texanus* is unknown, its most important host—raccoons—are known to be competent reservoirs, although only 35% as efficient as white-footed mice at infecting xenodiagnostic larvae (Fish and Daniels, 1990). Both *I. dentatus* and eastern cottontails are competent for transmission and maintenance of *B. burgdorferi* sensu lato (Telford and Spielman, 1989a); however, parasitism of *I. dentatus* on hosts other than birds and rabbits was low during our study and is unlikely to explain infections in these hosts. Lord et al. (1994) documented a site in Pennsylvania at which isolations of *B. burgdorferi* from mice were common, yet *I. scapularis* was not found, and suggested that some other mechanism besides transmission by a tick vector was responsible.

We detected three different *Borrelia* species within ticks and hosts (*B. burgdorferi*, *B. miyamotoi*, and *B. andersonii*), including a diverse assemblage of *B. burgdorferi* strains. The diversity of *B. burgdorferi* RST types along the coast suggests high rates of pathogen flow and/or multiple introduction events, rather than isolated founder effects. Similarly, all three RST types were found in alternative tick vectors or ear biopsies from sites beyond the invasion front. Studies of the population genetic structure of *B. burgdorferi* will be useful for determining the dynamics of *B. burgdorferi* in the presence and absence of *I. scapularis*, and how cryptic cycles affect *B. burgdorferi* infection prevalence and strain types in the bridging vector *I. scapularis*.

Intriguingly, a single *I. scapularis* larva dragged at an inland site was infected with *B. burgdorferi*. Sequencing of the IGS region identified this organism as RST 2, which is the most common type in the Midwest. Evidence for transovarial passage of *B. burgdorferi*, which would produce infected

questing *I. scapularis* larvae, is rare (but see Magnarelli et al., 1987 who found that transovarial transmission in *I. dammini* occurs at a variable rate). Although the single infected questing larva that we report herein is of little epidemiological significance, our finding was especially surprising considering the extremely low density of *I. scapularis* in the area. One possible explanation is that this infection was from spirochetes acquired during a partial larval bloodmeal on an infected host that groomed the larva off.

### Implications for Hypotheses

Our detections of *B. burgdorferi* shed light on our hypotheses about the emergence of the Lower Michigan *I. scapularis*/*B. burgdorferi* system. At four sites (C3, C4, I1, I3), we detected *I. scapularis* on mice after previous years of surveillance had failed to detect infestation on mice, and we interpret this as evidence for tick invasion into these sites. At site C4, no *B. burgdorferi* has yet been found, despite finding *I. scapularis* in both 2007 and 2008, supporting a tick-first process. At site C3, the first detection of *B. burgdorferi* coincided with that of *I. scapularis*, supporting a dual invasion process. At sites I1 and I3, *B. burgdorferi* was found in other sample types before detecting *I. scapularis*, supporting a spirochete-first process whereby *B. burgdorferi* is being maintained in cryptic transmission cycles. An alternative explanation is that a very low-density *I. scapularis* population—below our detection threshold—exists at I1 and I3. Such low-density *I. scapularis* populations would not normally be considered capable of maintaining *B. burgdorferi* (Madhav et al., 2004; Ogden et al., 2008).

### Implications for Detection of *I. scapularis*/*B. burgdorferi* Invasion

Studies of invasion most often are initiated once the invader has reached densities that have negative consequences (such as human disease) because resources for active surveillance and research are not usually appropriated in the absence of such impacts. Rarely are standardized investigations of wildlife undertaken in areas of no or low tick density that can provide a sensitive real-time warning system for invading ticks, spirochetes, and impending Lyme disease. One exception is Schulze et al. (1998), who reported a significant increase in human Lyme disease cases in 1990–1995 in Hunterdon County, NJ, that occurred after geographic expansion of ticks on deer in the county between 1981 and 1987. Falco et al.

(1995) monitored a 2.6-fold increase in questing *I. scapularis* nymphal density (from 13 to 34 nymphs/1,000 m<sup>2</sup>) in endemic Westchester Co., NY, from 1984 to 1991, which coincided with a 4.2-fold increase in reported human cases of infection. In contrast, at our study site C2, where nymphal drag data demonstrate invasion most clearly, densities increased from 0 to 5.3 nymphs/1,000 m<sup>2</sup> from 2004 to 2008 (Fig. 2.) Thus, we are assessing an invasion during its earliest stages, and human disease incidence is still very low (see below).

We found that prevalence of infestation of *I. scapularis* on white-footed mice provides a sensitive index of invasion (as did Lord et al., 1992); detection of ticks on mice generally preceded detection of ticks on drag cloths, alternative mammal species, and birds. Mouse sampling also was more useful than two other methods of surveillance for ticks and Lyme disease foci. Assessment of ticks on hunter-harvested deer in November across the study area was hampered by low densities of adult ticks in this early-stage invasion, and by a mismatch in the timing of the fall hunting season in relation to adult questing phenology in the invasion zone (their major activity peak is in the spring; S. Hamer, unpublished data). The efficacy of canine serosurveillance in the area was likely reduced by widespread use of anti-parasite prophylaxis on pet dogs living in the invasion zone (Hamer et al., 2009).

Alternative tick species and wildlife hosts enabled detection of *B. burgdorferi* in the apparent absence of *I. scapularis*. Detection of cryptically cycling *B. burgdorferi* decouples its invasion from that of *I. scapularis*, and may hasten *B. burgdorferi* transmission dynamics among an invading *I. scapularis* ticks. Cryptically cycling *B. burgdorferi* thus may decrease the time lag between first detection of *I. scapularis* and first detection of *B. burgdorferi*-infected *I. scapularis*.

### Study Limitations

The results we present are based on sampling within the May and June period only, which does not include all months of blacklegged tick activity. The most commonly cited *I. scapularis* phenology—based on studies in Westchester Co., NY (Fish, 1993)—is characterized by bimodal peaks in adult activity in the spring and fall and nymphal activity in the early summer, which precedes peak larval activity in the late summer. Gatewood et al. (2009) noted that whereas the Northeast is generally characterized by distinctly separate peaks in nymphal and larval activity in

June and August, respectively, as indicated above, the Midwest is characterized by synchronous feeding of nymphs and larvae in June through July, with a much smaller late-season peak in larval activity. Whereas we present 5 years of data from May and June only (early summer), all our sites also were sampled during July–August (late summer) in at least one year with similar rodent trapping and drag sampling effort (unpublished data). At all sites where we found *I. scapularis* in the late summer, this species also was found in the early summer period reported here. Furthermore, in 2008–2009 we conducted a longitudinal study of *I. scapularis* phenology at monthly intervals at site C1 (unpublished data). Larval infestation of mice was greatest in June (83.8%), followed by a decline (44.1%, 48%, 24.5%, and 19.4% monthly infestation prevalence in July–October, respectively), and nymphal infestation followed a similar trend (24.3%, 8.5%, 2.0%, 2.0, and 0% in June–October, respectively). These patterns support the synchronized feeding reported by Gatewood et al. (2009) and are similar those reported by Godsey et al. (1987) in Wisconsin and Strand et al. (1992) in Michigan’s Upper Peninsula. Thus, we conclude that our sampling focused on the period during which we had the best chance of detecting infested mice each year and that this period was appropriate for answering our research questions.

A longer duration of sampling at all sites would have provided greater sensitivity of detection of stable *I. scapularis* populations. Whereas neither our data nor that of Gatewood et al. (2009) show significant larval activity in the late summer, late summer larval activity of greater (Northeast) or equivalent (Midwest) magnitude to early summer activity has been reported (Daniels et al., 1996; Jones and Kitron, 2000; Kitron et al., 1991). In the Northeast, the early peak in May–June represents the remainder of the cohort of larvae that hatched the previous July and successfully overwintered, whereas the second peak of higher magnitude in August represents the subsequent cohort of newly hatched larvae from recently laid eggs by adults that were active in spring as well as the previous fall (after ovipositional diapause; Daniels et al., 1996). In the Midwest, the separate peaks observed by some researchers at some sites are posited to result from the separate oviposition periods of the spring and fall adults (Jones and Kitron, 2000; Kitron et al., 1991), whereby the early larvae may result from oviposition by fall adults and the late summer larvae result from oviposition by spring adults. Furthermore, there has been a suggestion that this bimodality may be more pronounced in newly establishing

populations (Jones and Kitron, 2000). Therefore, if black-legged tick invasion in Michigan is driven by adult ticks moving in during the spring (e.g., on deer), our sampling may miss the resulting larvae emerging late that first summer.

Assaying ticks of all stages and species removed from hosts increased our capability to detect the presence of *B. burgdorferi* at a site compared with assaying *I. scapularis* or host tissue alone. Our protocol, however, did not maximally allow us to detect *B. burgdorferi* infection because we did not assay all ticks removed from hosts. We selected a random subset of three per species/stage/sex combination to assay for infection, sometimes resulting in over ten ticks tested from some hosts that were infested by multiple tick species and stages. Given limited resources, this protocol allows us to gather infection data from ticks removed from a greater number and diversity of hosts as well as to avoid overrepresenting heavily parasitized animals in the overall results of tick infection. Testing of all ticks from such animals would have increased the odds of both detecting a positive tick on a given animal and detecting different genospecies that coinfect the same vertebrate host (although that latter was not a main study objective). Although comprehensive tick testing may have resulted in a finer temporal resolution of invasion dynamics, we do not believe it would have altered our qualitative conclusions.

### Implications for Disease and Prevention

Data collected from careful study of the underlying ecology and transmission of zoonotic pathogens within animal reservoirs before and during invasion can provide an early warning of increasing disease risk to human and companion animals. Cryptic cycling of *B. burgdorferi* does not imply human risk of Lyme disease in these areas but may hasten the establishment of transmission cycles that involve *I. scapularis*. Such cycling also may introduce different strains of *B. burgdorferi* to *I. scapularis*, which may lead to variable clinical manifestations (Wormser et al., 1999).

The tick/spirochete invasion we document in this paper is associated with a significant recent increase in confirmed cases of human Lyme disease within the Southwest/Western Michigan zone of invasion. Incidence in this 14-county region increased during the period from 1996 (when Lyme disease became reportable in Michigan) to 2008 from 0.16 cases per 100,000 people to a peak incidence of 0.63 ( $R^2 = 0.28$ ; 1-tailed  $P = 0.03$ ; E. Foster, Michigan Department of Community Health, personal

communication). Incidence remains substantially lower than the average annual incidence in the ten endemic states from which > 93% of human Lyme disease across the United States is reported (average of 29.2 cases per 100,000 population for 2003–2005; Bacon et al. 2008). In contrast with the significant peridomestic exposures that occur in the Northeastern United States, recreational exposure is probably common in the Michigan invasion zone, which is dominated by recreational areas, camps, vacation homes, and rural communities. Recreational exposure may produce low reported disease incidence locally, because recreational visitors will return home (often to other counties or states) before developing symptoms and seeking medical attention. Spatial epidemiology and trace-back of human Lyme disease cases reported elsewhere in Lower Michigan would help shed light on this possibility.

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Supplementary 1. Bird-tick associations and infestation prevalences on the inland and coastal transects in Lower Michigan, May-June, 2004-2008. L = larva; N = nymph; A = adult. Number of birds carrying larvae/nymphs/adults is indicated with the percent of birds infested with at least one tick of any life stage in parenthesis. All ticks were tested for *Borrelia burgdorferi*; those birds with infected ticks are indicated in Table S1.

Species	Total		Coastal			Inland		
	no.	no.	<i>I. scapularis</i>	<i>I. dentatus</i>	<i>H. leporispalustris</i>	no.	<i>I. dentatus</i>	<i>H. leporispalustris</i>
American goldfinch	65	22				43		
American redstart	22	22				0		
American robin	46	20	4/8/0 (40)	1/1/0 (10)	2/2/0 (15)	26	9/11/0 (53.8)	0/2/0 (7.7)
Baltimore oriole	9	0				9		
black-billed cuckoo	1	0				1		
black-capped chickadee	30	11				19	2/0/0 (10.5)	
black-throated green warbler	1	1				0		
blue jay	1	0				1		
blue-winged warbler	1	0				1		
brown thrasher	3	0				3	2/1/0 (66.7)	
brown-headed cowbird	8	2		1/1/0 (25)		6		
cedar waxwing	9	2				7		
chipping sparrow	40	33	0/2/0 (6.1)			7		
common grackle	2	1				1		
common yellowthroat	53	9		0/0/1 (11.1)		44	3/0/0 (6.8)	
Connecticut warbler	1	1				0		
downy woodpecker	6	1				5		
Eastern bluebird	6	3				3		
Eastern kingbird	2	1				1		
Eastern phoebe	4	3		1/0/0 (33.3)		1		
Eastern towhee	2	1	0/1/0 (100)			1		
Eastern wood-pewee	8	3				5		
<i>Empidonax</i> flycatchers	14	6				8		
European starling	1	0				1		
field sparrow	8	2				6	1/0/0 (16.7)	
gray catbird	89	13				76	4/3/0 (6.6)	
gray-cheeked thrush	1	1				0		
great crested flycatcher	1	0				1		
hairy woodpecker	1	1				0		
house finch	3	3				0		
house wren	13	9		0/1/0 (11.1)		4	2/0/0 (50)	
indigo bunting	22	21	0/1/0 (4.8)			1		
mourning dove	1	0				1		
Northern cardinal	16	7	1/1/0 (14.3)	0/0/1 (14.3)		9	2/0/0 (22.2)	
Northern waterthrush	1	0				1		
orchard oriole	2	0				2		
ovenbird	1	1				0		
pileated woodpecker	1	1				0		
red-eyed vireo	15	15				0		
red-winged blackbird	80	0				80	13/0/0 (16.3)	0/1/0 (1.3)
rose-breasted grosbeak	7	2				5	1/0/0 (20)	
song sparrow	52	9		1/1/0 (11.1)		43	13/6/0 (32.6)	1/2/0 (7.0)
swamp sparrow	4	0				4	1/0/0 (25)	
Tennessee warbler	1	1				0		
Trail's flycatcher	4	0				4		
tree swallow	3	0				3		
tufted titmouse	2	2				0		
unknown	2	0				2		
warbling vireo	2	0				2		
white-crowned sparrow	2	0				2		
Wilson's warbler	2	0				2		
wood thrush	2	0				2	2/1/0 (100)	
yellow warbler	65	7				58	1/1/0 (3.4)	
yellow-breasted chat	1	0				1	1/0/0 (100)	
yellow-sided flycatcher	1	0				1		
yellow-throated vireo	1	0				1		
<b>All species</b>	<b>741</b>	<b>237</b>	<b>5/13/0 (5.5)</b>	<b>4/4/1 (3.4)</b>	<b>2/2/0 (1.3)</b>	<b>504</b>	<b>57/23/0 (12.9)</b>	<b>1/5/0 (1.2)</b>

Supplementary 2. Infection prevalence of each species of tick removed from mammalian and avian hosts in lower Michigan, May-June, 2004-2008. Infection prevalence is presented as the percent of ticks that tested positive with the sample size of ticks tested is in parenthesis. Larval infection is reported as the minimum infection prevalence. LL = larvae; NN = nymph; AA = adult. Regarding birds, only those species with positive ticks are listed below; a complete list of all 56 bird species and associated ticks is provided in Table S1.

Host species	Transect	Stage	<i>D. variabilis</i>	<i>I. scapularis</i>	<i>I. texanus</i>	<i>I. cookei</i>	<i>I. dentatus</i>	<i>H. leporispalustris</i>	<i>I. marxi</i>	<i>A. americanum</i>
Northern short-tailed shrew	Coastal	LL		0 (1)						
Virginia opossum	Inland	LL				0 (2)				
		NN			0 (1)	0 (1)				
		AA	1.1 (91)		0 (1)	0 (1)				
	Coastal	LL		0 (52)						
		NN		6.9 (29)		0 (5)				
		AA								
Southern flying squirrel	Coastal	LL	0 (3)		100 (1)					
		NN		0 (2)						
		AA	0 (3)						50 (2)	
Striped skunk	Coastal	LL				0 (19)				
		NN		0 (3)		0 (1)				
		AA				0 (2)				
Meadow vole	Coastal	LL	0 (1)							
Long-tailed weasel	Inland	LL	0 (1)			0 (1)				
		NN				0 (2)				
White-footed mouse	Inland	LL	0.7 (2116)	0 (5)			0 (1)			
		NN	6.6 (106)	50 (2)						
	Coastal	LL	2.4 (716)	5.4 (705)	0 (1)					
		NN	17.6 (74)	37.8 (82)	0 (1)					
Raccoon	Inland	LL	0 (1)		2.4 (41)	0 (1)				
		NN	0 (7)		2.6 (78)	0 (6)				
		AA	1.4 (209)		2.4 (83)	0 (2)				
	Coastal	LL	0 (1)	0 (5)	0 (158)					
		NN	0 (1)	9.1 (22)	0 (94)					
		AA	2.4 (164)	0 (2)	2.3 (88)	11.1 (9)				0 (2)
Eastern gray squirrel	Coastal	LL	0 (2)	25 (4)						
		NN		40 (5)						
		AA	0 (6)							
Fox squirrel	Coastal	LL		0 (2)						
		NN		0 (1)						
Eastern cottontail	Inland	AA	0 (3)				12.9 (31)		0 (2)	
	Coastal	LL		66.7 (3)						
		NN	0 (1)						0 (3)	
		AA					0 (2)		0 (6)	
Eastern chipmunk	Inland	LL	5 (20)							
		NN	0 (2)							
	Coastal	LL	0 (2)	9.8 (82)	0 (1)					
		NN		26.2 (65)						
Red squirrel	Inland	NN	0 (1)						0 (1)	
		AA			0 (1)				0 (3)	
	Coastal	LL		0 (1)						
		AA			0 (3)					
Meadow jumping mouse	Inland	LL	0 (12)							
		NN	0 (1)				0 (1)			
	Coastal	LL		0 (1)						
		NN		0 (2)						
		AA					0 (1)			
American Robin	Inland	LL					2.9 (35)			
	Coastal	LL		7.7 (13)						
		NN		37.5 (16)					50 (2)	
Northern cardinal	Coastal	LL		100 (1)						
		NN		100 (1)						
Brown-headed cowbird	Coastal	LL					100 (1)			
Song sparrow	Inland	LL					5.1 (39)			
		NN					12.5 (8)			
Red-winged blackbird	Inland	LL					2.1 (47)			