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ABSTRACT The risk of Lyme disease for humans in the eastern United States is dependent on the density of host-seeking *Ixodes scapularis* Say nymphal stage ticks infected with *Borrelia burgdorferi*. Although many local and regional studies have estimated Lyme disease risk using these parameters, this is the first large-scale study using a standardized methodology. Density of host-seeking *I. scapularis* nymphs was measured by drag sampling of closed canopy deciduous forest habitats in 95 locations spaced among 2° quadrants covering the entire United States east of the 100th meridian. Sampling was done in five standardized transects at each site and repeated three to six times during the summer of 2004. The total number of adults and nymphs of the seven tick species collected was 17,972, with 1,405 nymphal *I. scapularis* collected in 31 of the 95 sites. Peak global spatial autocorrelation values were found at the smallest lag distance (300 km) and decreased significantly after 1,000 km. Local autocorrelation statistics identified two significant high-density clusters around endemic areas in the northeast and upper Midwest and a low-density cluster in sites south of the 39th parallel, where only 21 nymphs were collected. Peak nymphal host-seeking density occurred earlier in the southern than in the most northern sites. Spatiotemporal density patterns will be combined with *Borrelia* prevalence data as part of a 4-yr survey to generate a nationwide spatial risk model for *I. scapularis*-borne *Borrelia*, which will improve targeting of disease prevention efforts.

KEY WORDS *Ixodes scapularis* nymphs, *Borrelia burgdorferi*, spatial clustering, phenology, risk mapping

THE BLACKLEGGED TICK, *Ixodes scapularis* Say is the principal vector for *Borrelia burgdorferi*, the causative agent of Lyme disease in North America. The number of Lyme disease cases rose from 17,029 cases in 2001 to 23,763 in 2002, a 40% increase (CDC 2004a), with a slight reduction in 2003 (21,273 cases) (CDC 2004b). Efforts to reduce the risk of Lyme disease are more cost effective when targeted toward areas and times of the year associated with a high frequency of contact between humans and host-seeking *I. scapularis*, particularly those infected with *B. burgdorferi*. The nymphal stage of *I. scapularis* is the only stage with a significant role as a vector for *B. burgdorferi* in North America (Stafford et al. 1998, Falco et al. 1999, Piesman 2002). Because of their small size, nymphs often escape detection long enough to transmit *B. burgdorferi* (Piesman 1987, Falco et al. 1996) and nymphal host-seeking reaches its peak in spring and early summer, which coincides with increased human outdoor activity (Fish 1993). Drag sampling provides the most sensitive measure of potential contact between ticks and humans (Falco and Fish 1992, Schulze et al. 1997, Daniels et al. 2000). Therefore, mapping the spatial and temporal distribution of host-seeking nymphs collected by drag sampling provides an accurate estimate of human contact with potentially infected nymphs. Combined with tick infection prevalence data, an entomological risk index (ERI) (Mather et al. 1996a) can be calculated from nymphal density estimates. ERI constitutes an accurate and sensitive measure of risk to humans that, when mapped at a national scale, can provide an invaluable tool for targeting prevention efforts (Fish and Howard 1999).

The construction of an accurate map showing the spatial distribution and relative abundance of *I. scapularis* in the United States has been limited by passive and nonstandardized collection methods (Dennis et al. 1998). Consequently, many of the cited reports of *I. scapularis* collections in areas previously free of Lyme disease (reviewed in Ginsberg 1993) may represent either new invasions by *I. scapularis* or overlooked populations. The only previous national mapping efforts have been based on a county-level map of the distribution of *I. scapularis* and *I. pacificus* based on

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indirect measurements of presence or abundance, including questionnaires to acarologists, health officials, and Lyme disease researchers, literature surveys, and review of the 1907–1995 National Tick Collection data (Dennis et al. 1998). To generate this map, counties were classified as having either established populations (at least six ticks or >1 life stage identified), reported occurrence (at least one tick of any life stage identified) or absent populations (no positive collection data). Fish and Howard (1999) improved this map by using a neighborhood analysis procedure that smoothed absent data and minimized reporting gaps. They also combined it with indirect measures of infection prevalence in ticks and human exposure to produce the National Lyme Disease Risk Map, created for the Centers for Disease Control and Prevention (CDC) Advisory Committee for Immunization Practices. The recommendations for vaccination against Lyme disease were based entirely upon the geographic distribution of Lyme disease risk delineated in this map. Using data from Dennis et al. (1998), Brownstein et al. (2003) developed a spatially predictive logistic model for *I. scapularis* in the United States using ground-observed environmental data to predict the probability of established *I. scapularis* populations.

Standardized efforts to measure risk have been conducted at the state level, where human risk for Lyme disease has been quantified based on vector distribution (Drew et al. 1988, Novak et al. 1991, Schulze et al. 1991, Kitron et al. 1992, Daniels et al. 1993, Bartholomew et al. 1995, Pinger et al. 1996, Riehle and Paskewitz 1996, Walker et al. 1998, Guerra et al. 2001) and human case reports (Glavanakov et al. 2001). In studies including both tick surveys and human case surveillance and/or canine serosurveys, these two measurements have been found to be correlated (Rand et al. 1991, White et al. 1991, Daniels et al. 1993, Nicholson and Mather 1996, Kitron and Kazmierczak 1997, Stafford et al. 1998, Guerra et al. 2001), although this has not always been the case (Mather et al. 1996b, Daniels et al. 1998, Brownstein et al. 2005). Inconsistencies between both types of measurements could be because of frequent misdiagnosis of Lyme disease (Barbour and Fish 1993, Steere et al. 1993) or differences in human behavior that change the probability of human exposure to infected ticks (Brownstein et al. 2005). The problem of risk mapping for Lyme disease is also complicated by the fact that the distribution of *I. scapularis* is expanding (Madhav et al. 2004). Areas of new invasion are quickly followed by human case reports (Lastavica et al. 1989, White et al. 1991).

Here, we present the first year results of a multiyear project to produce a spatial risk model for *I. scapularis*-borne *Borrelia* throughout the known range of *I. scapularis* in the United States. Patterns of spatial clustering and host-seeking phenology of *I. scapularis* nymphs are presented. Future project objectives include combining these results with data on *B. burgdorferi* infection prevalence and genotype to estimate risk. This project promises to provide a reliable national risk map that can be used as a guide to geographically prioritize prevention efforts. It will provide an improved delineation of risk over the Fish and Howard (1999) National Lyme Disease Risk Map, because it will include both host-seeking nymphal tick density and infection prevalence.

Materials and Methods

Study Area. The study area encompassed the portion of the United States east of the 100th meridian, which includes the known distribution of *I. scapularis.* Ninety-five sites were sampled throughout the study area. To distribute the sites uniformly, a grid composed of 95 2° (≈160 by 160 km) quadrants (Fig. 1) was overlaid on a map of the United States and one site within each of the quadrants was randomly selected for sampling. All data sets used in the analysis were projected to an equidistant conic projection with 34 and 40° N as standard parallels to increase the accuracy of the distance measurements for the spatial analyses. The sampling sites were areas of closed canopy deciduous forest within state parks, state forests, or other public access natural areas. Public areas were identified from the Census 2000 Tiger line files. To select which parks to sample, the following procedure was followed. First, the area of deciduous or mixed deciduous forest within each park was calculated from the National Land Cover Characterization database produced by the United States Geological Survey. This is a 21-class land cover classification scheme based on Landsat Thematic Mapper satellite data acquired from the early to mid-1990s, including both leaf-on and leaf-off scenes (http://landcover.usgs.gov/natllandcover.asp). The 30-m resolution of this imagery allowed for calculation of the area of deciduous forest cover even in small state parks. Within each quadrant, all parks were ranked according to total forested area, and a site was randomly selected from among the top 20%. Where suitability models for *I. scapularis* establishment were available, they were considered for site selection. Site selection was restricted to areas assigned a probability of containing habitat suitable for *I. scapularis* establishment of >50% according to the Brownstein et al. (2003) model in the Northeast and the Guerra et al. (2001) model in Wisconsin and Illinois.

Sampling Protocol. Sampling was done between 19 May and 27 August 2005. Each site was scheduled to be sampled four to six times over the summer, although this was not always possible (see Results). Nymphal densities were estimated at each site by dragging a 1-m^2 cloth over a fixed distance (Daniels and Fish 1990). Maps of the parks were used to select five areas for sampling (Fig. 2). From the start of a trail in each area, a random number was drawn to determine the number of steps to be walked to the start of a transect. The geographic coordinates of the transect start relative to the World Geodetic System 1984 datum were recorded using a handheld Global Positioning System (GPS) receiver (eTrex, Garmin International Inc., Olathe, KS) with a position accuracy between 3 and 5 m. Another random number was drawn to determine the transect orientation (angle)

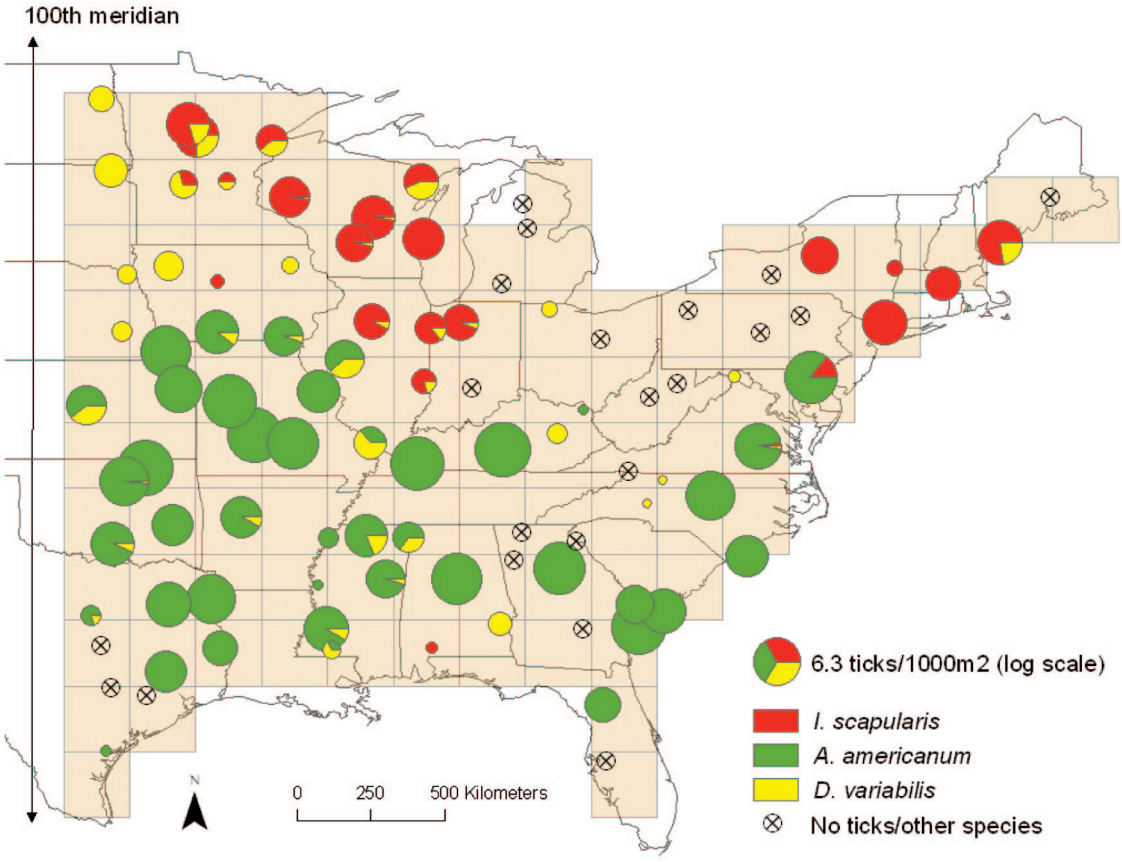

Fig. 1. Density per 1,000 m^2 (log scale) of the most abundant species of ticks (nymphs and adults pooled) collected in each of the 95 study sites. The grid used to select the sampling sites is displayed in the background as well as the 100th meridian, western limit of the study area.

relative to the trail. Two parallel 100-m transects were dragged in each of five locations, for a total sample area of 1,000 m^2 per site per visit. The cloth was inspected every 20 m, and all nymphs and adults from each transect were put into bar-coded vials with 70% ethanol. Sampling was performed on rain-free days, avoiding early morning and midday hours to minimize the potentially confounding effects of heavy dew and extreme heat on sampling efficiency. Ticks from each transect/date were identified to species and stage and sorted into separate bar-coded vials.

Temperature, relative humidity, and atmospheric pressure were recorded upon arrival and departure from each sampling site using a digital weather meter (Kestrel 4000, Nielsen-Kellerman, Boothwyn, PA). Time was recorded at the beginning and end of each transect. Saturation deficit, an integrated measure of the drying power of the atmosphere, was calculated according to Randolph and Storey (1999).

Data Storage and Manipulation. Data were stored in a MySQL database management system. The bar code on each tube was linked in the database to all other data related to that collection (e.g., environmental data and geographic location). For analysis, the online database was exported to ArcGIS 9 Desktop, an integrated collection of geographic information system (GIS) software products (ESRI Inc., Redlands, CA). ArcInfo was used to display and manipulate the data and ArcToolbox spatial statistics tools for spatial analysis. Further statistical analyses were performed using Stata/SE 8.0 (Stata Co., College Station, TX).

Statistical Analysis. Moran's *I* (Cliff and Ord 1973, 1981) was used to measure whether *I. scapularis* density was spatially clustered. This index measures spatial autocorrelation, that is, the degree of interdependence between values of a variable at different geographic scales. As distance increases, the value of Moran's *I* is expected to decrease, because increasing distance diminishes correlation. Moran's *I* statistic was calculated as follows:

$$I(d) = \frac{N \sum_{ij} w_{ij}(d)(x_i - \bar{x})(x_j - \bar{x})}{\left(\sum_{ij} w_{ij}(d)\right)\left(\sum_{i} (x_i - \bar{x})^2\right)} \quad i \neq j \qquad [1]$$

where N is the total number of sampling sites; x_i and x_j are the ln(*I. scapularis* mean density per site + 0.5) in sampling sites i and j, respectively; $\bar{x}$ is the sample mean for all x values; d is the fixed distance interval;

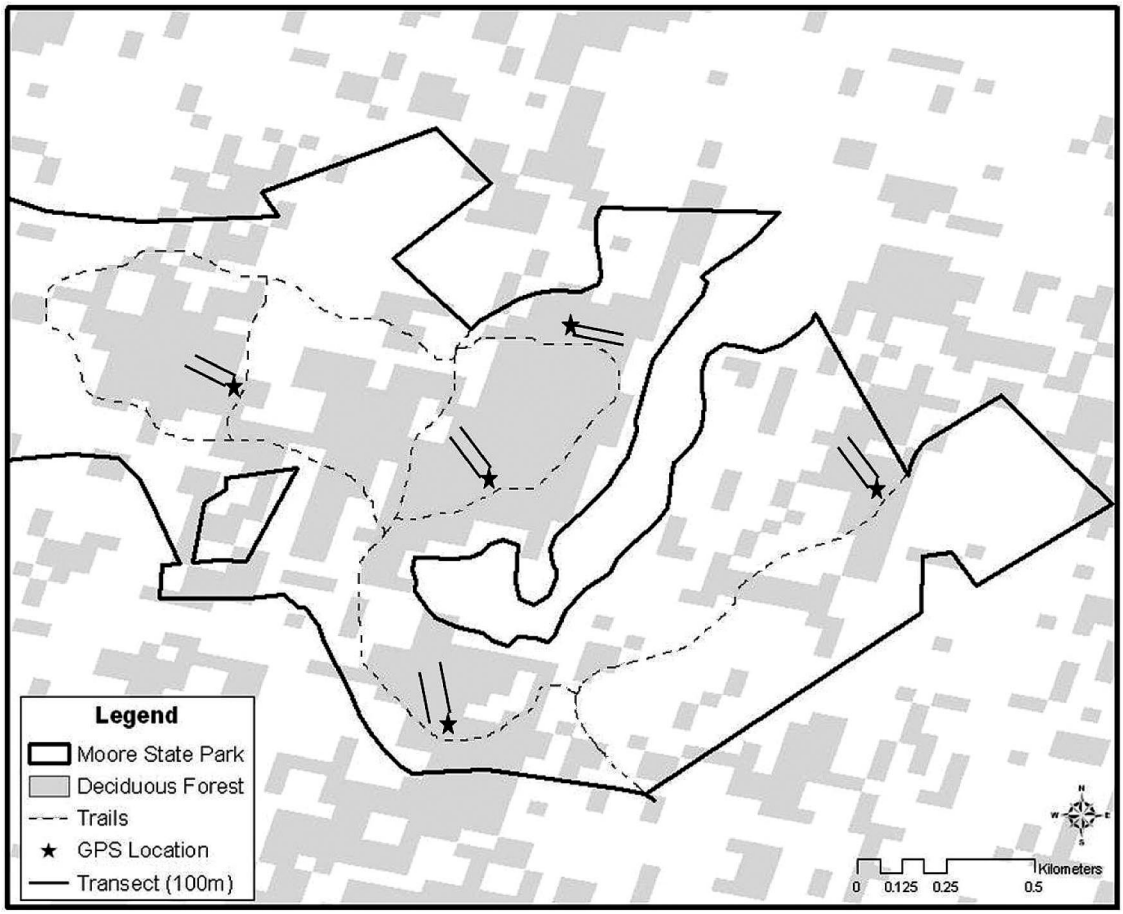

Fig. 2. Illustration of sampling protocol within each sampling site. Five trails were identified in the five largest areas of deciduous forest in the park. In each trail two parallel 100-m transects were dragged for ticks and the start of each transect was recorded with a GPS. Both the number of steps to the start of the transect and the angle were determined using a random number generator.

and w_{ij} is a weight based on the inverse of the distance between sites. The logarithmic transformation reduced skewness and kurtosis of the tick distribution although normality was not achieved.

Moran's I ranges from -1 to 1 and equals 0 when there is no spatial autocorrelation. Positive values indicate that variation in tick densities is dependent on the distances between sites; sites with high tick density are clustered in space as are those with low tick densities, with higher values indicating a stronger relationship. A spatial correlogram, a series of Moran's I measurements evaluated at increasing distances between sampling sites, was used to determine the distance at which spatial effects are maximized. The distance intervals were set at 300 km to ensure that all first neighbors were included in the first lag.

Moran's I does not identify the specific locations of pockets of clustering. Local indicators of spatial autocorrelation (LISA) (Anselin 1995) provide a way to identify patterns at a local scale. Local spatial clusters are defined by Anselin (1995) as sets of continuous locations for which the LISA is significant. The G_i^* LISA (Getis and Ord 1992, Ord and Getis 1995) was used to identify the locations of clusters of sites with high and low *I. scapularis* nymphal density. G_i^* was calculated for all sampling sites as follows:

$$G_i^*(d) = \frac{\sum_{j=1}^{n} w_{ij}(d)\, x_j}{\sum_{j=1}^{n} x_j} \quad \text{for all } j \qquad [2]$$

where i is the focal sampling site, x_j is the ln(*I. scapularis* mean density per site + 0.5) in sampling site j (including the focal sampling site x_i), d is the fixed distance interval, and w_{ij} is a weight that is 1 when sites are within distance d of the focal point and 0 otherwise.

The null hypothesis states that the sum of values at all j sites within radius d of i is equal to that expected by chance given all the values in the entire study area. G_i^* was calculated for the same distance classes as

Table 1. Numbers of adults and nymphs of all tick species collected during the study

Species	Adults	Nymphs	Total
I. scapularis	135	1,405	1,540
Dermacentor variabilis (Say)	492	9	501
Amblyomma americanum (L.)	1,580	14,341	15,921
Amblyomma maculatum Koch	4	1	5
Haemaphysalis leporispalustris (Packard)	3	0	3
Ixodes dentatus Marx	1	0	1
Ixodes muris Bishopp & Smith	1	0	1
Total for all species:	2,216	15,756	17,972

Moran's *I*. To determine whether clustering was significant, G_i was redefined as a standard variable, for which the expected value in the absence of clustering is 0. However, significance of the G_i^* (d) about the selected focus site is not clear-cut, especially when global autocorrelation is significant and the variable distribution is not normal. Thus, we used this statistic only as an exploratory tool.

Nymphal *I. scapularis* Host-Seeking Phenology. The seasonal pattern of host-seeking nymphal density is expected to fit a polynomial function, with an increase during late spring, a peak in early June, and a decrease during midsummer (Fish 1993). However, in those sites where collections started at the time of peak density, or soon after, a negative linear function may result in a better fit. For those sites visited four or more times, with five samples per visit and a total of >20 nymphs collected, the fit to linear and quadratic regressions were compared, and the function that resulted in a better fit was plotted. Higher polynomials were not considered appropriate given the small sample size. Twenty nymphs were arbitrarily defined as the minimum number to detect phenological patterns based on examination of the plots.

Results

Total Tick Collection. The total number of adults and nymphs of all seven tick species collected was 17,972 (Table 1); the total area drag-sampled was 431,000 m^2 in 38 states. The relative proportions of the different species varied spatially, with *I. scapularis* dominating in the Northeast and upper Midwest, *A. americanum* dominating in the south, and *D. variabilis* being widely distributed (Fig. 1). A cautionary point is that sampling was done solely in deciduous forests, excluding grassy meadows, old field–forest ecotones, and artificial habitations, where *D. variabilis* would have been the dominant species found (Sonenshine 1993), thus biasing the sampling toward the other two species. Analyses of tick species other than *I. scapularis* will be reported elsewhere.

Nymphal *I. scapularis* Density. In total, 1,405 *I. scapularis* nymphs were collected in 31 of the 95 sites studied. For comparison with other studies, sites were classified into terciles, based on nymphal *I. scapularis* density: low density, when fewer than 0.7 nymph per 1,000 m^2 was collected; intermediate density, when between 0.7 and 8.2 nymphs per 1,000 m^2 were collected; and high density, when between 8.2 and 48 (Fig. 4). The average density of nymphs in positive sites was 9.9 nymphs per 1,000 m^2. Highest densities were observed in the northeast and mid-Atlantic regions (18.2 per 1,000 m^2), with the highest values in Westchester County, New York, and southern Maine; and in the upper Midwest (11.9 per 1,000 m^2), especially in Wisconsin and Minnesota (Fig. 4). Only 21 *I. scapularis* nymphs were collected in the 16 states south of the 39th parallel despite 223,400 m^2 of drag sampling (0.09 per 1,000 m^2) (see location of this parallel in Fig. 4): seven nymphs in Virginia, one in South Carolina, one in Georgia, one in Alabama, five in Oklahoma, and six in Missouri.

Moran's *I* values indicated significant spatial clustering of nymphal *I. scapularis* density at all distances examined in the correlogram (Fig. 3). Peak Moran's *I* values were found at the smallest lag distance (300 km) and declined to almost 0 at 1,000 km, which implies that there was little association between tick density at sites located >1,000 km away. The local spatial statistic G_i^* allowed for examination of the actual location and approximate scale of both the high- and low-density clusters, at successively large distances (Fig. 4). *Gi** values at the 300-km distance class identified two significant positive clusters that closely traced the empirical density values. Only one negative site in eastern Pennsylvania showed significantly positive *Gi** values, and all positive *I. scapularis* sites, except the "southern" sites and a site in north central Iowa, showed positive *Gi** values. A significant low-density cluster was identified in the southern part of the study area when larger distances were used to calculate *Gi** (900 and 1,200 km). At these larger lag distances, nonsignificant low-density sites were either located between high-density sites in the north and the low-density cluster in the south or near the map border, where they did not reach significance because of the smaller number of neighbors.

The mean number of nymphal ticks collected per site in positive sites (n= 31) was significantly and negatively correlated with mean temperature (Spear-

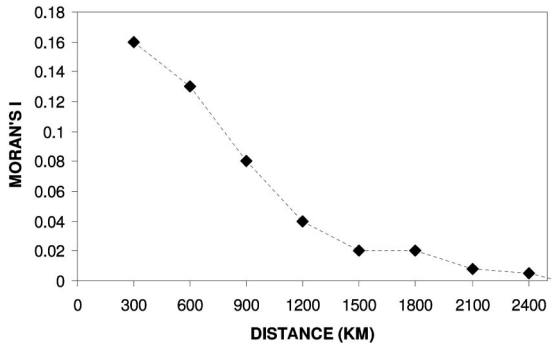

Fig. 3. Spatial correlogram showing the dependence of Moran's *I*, the spatial autocorrelation coefficient, on the distance between sampling sites, grouped into 300-km categories. Spatial autocorrelation exceeds zero significantly for all lags.

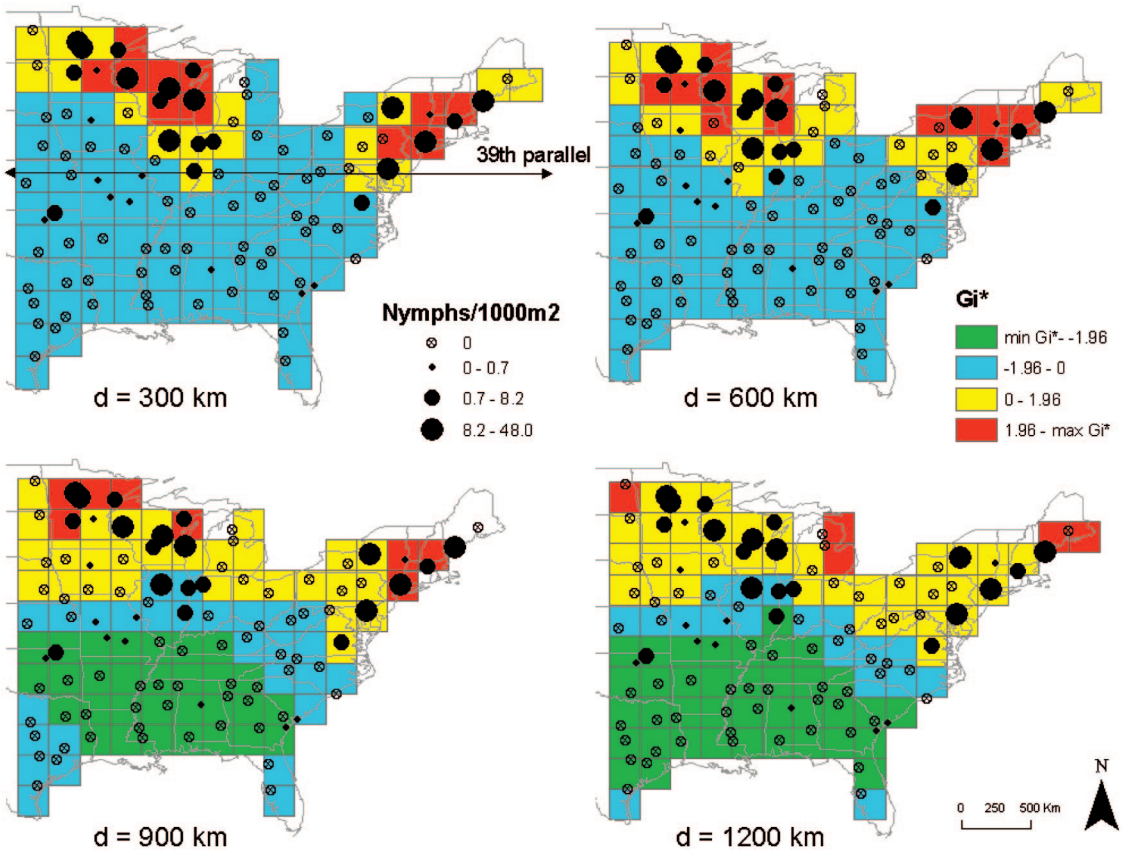

Fig. 4. Mean density of host-seeking *I. scapularis* nymphs per 1,000 m^2 dragged in each site. Density categories are no ticks (circle with cross), low (0–0.7 nymph per 1,000 m^2), moderate (0.7–8.2 nymphs per 1,000 m^2), and high (8.2–48 nymphs per 1,000 m^2). The four subfigures show clustering patterns of nymphal *I. scapularis* density based on the Getis-Ord Gi* statistic at different distances (d). Quadrants were colored according to the Gi* value for the sampling site within them. Red quadrants (Gi* higher than 1.96) represent significant high-density clusters, green quadrants (Gi* lower than −1.96) represent significant negative clusters, yellow areas (Gi* between 0 and 1.96) are high-density clusters that are not statistically significant, and light blue areas (Gi* between −1.96 and 0) are low-density clusters that are not statistically significant.

man's rho = −0.64, $P < 0.001$) and saturation deficit (Spearman's rho = −0.72, $P < 0.001$) at the site and positively correlated with latitude (Spearman's rho = 0.53, $P < 0.01$). It was not correlated with either mean relative humidity or time of collection.

Nymphal *I. scapularis* Phenology. Most sites were sampled three or more times during the season, except six sites visited twice and one site visited once, because of rainy weather. The phenology of nymphal *I. scapularis* in those sites with four or more visits and a total of >20 nymphs collected ($n = 12$) is shown in Fig. 5A for the northeast and in Fig. 5B for the upper Midwest. Nymphal density showed a better fit (higher R^2) to a quadratic than a linear regression at four of the most northern sites (Paul Bunyan, Foot Hills State forests in the upper Midwest, Crescent Beach, and Verona Beach State Parks in the northeast). Data from all other sites showed a better fit to a linear regression, likely because only the declining part of the phenological curve was captured in these sites. Peak nymphal density (defined as the maximum density recorded) occurred between 4 June and 19 July 2004 in the four "northern" sites and before 22 June in the "southern" sites.

Discussion

Two statistically significant clusters of high-density host-seeking nymphal *I. scapularis* were identified along coastal areas in the northeastern United States and in an upper Midwest area, including Wisconsin and Minnesota. These are known *I. scapularis* endemic areas, but previous studies did not use a standardized sampling protocol and spatial analytical tools to quantitatively assess the locations and extents of high-risk areas based on host-seeking nymphs. In addition, our study includes the southern states, where host-seeking *I. scapularis* nymphs are known to be difficult to collect (Piesman 2002). Mapping of statistically significant low-density host-seeking nymphal clusters in the south defined transitional areas between northern and southern clusters that require further resolution.

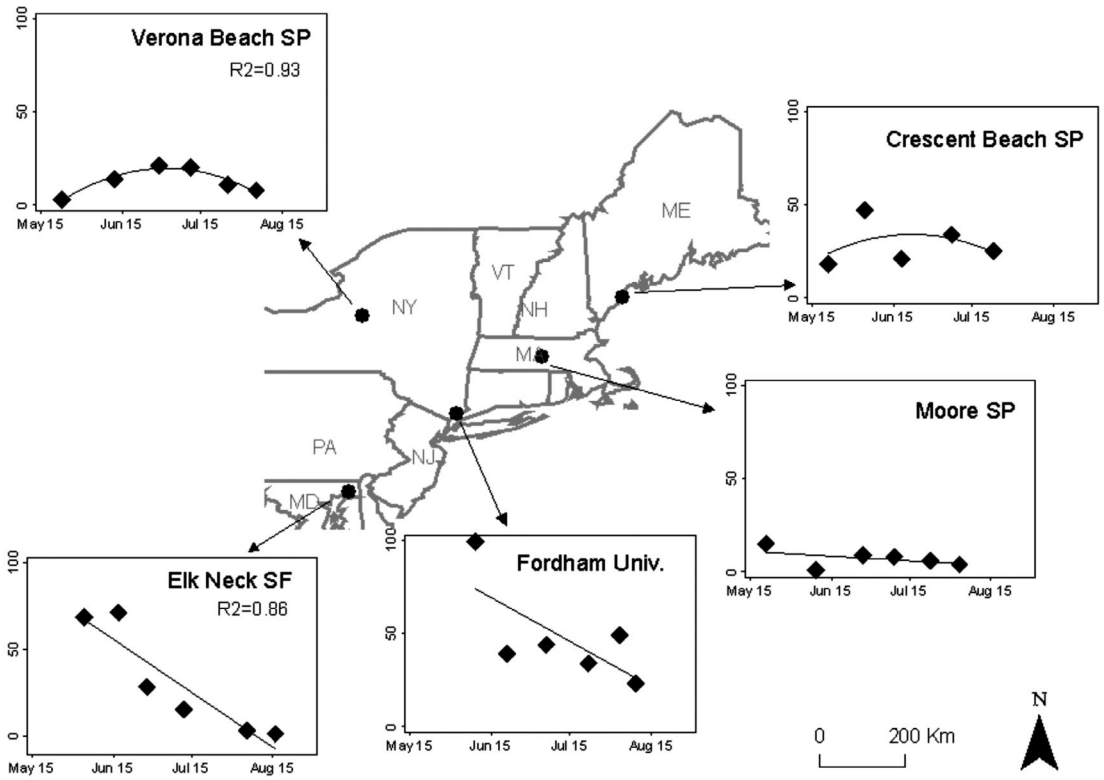

Fig. 5A. Density of *I. scapularis* nymphs per 1,000 m^2 (*y*-axis) collected in each visit (date in *x*-axis) from northeast sampling sites. The best fitting curve (linear or quadratic) is shown for each collection. R^2 values are shown for those curves that had a significant fit ($P < 0.05$).

The observed patterns of spatial dependence can provide clues to the processes underlying the current distribution of host-seeking nymphal *I. scapularis.* In the northeast and upper Midwest, generally monotonic decline of Moran's *I* with distance indicates clinal variation. The range of *I. scapularis* is known to be expanding (Spielman et al. 1985, Dennis et al. 1998). Significant high-density clusters were identified around the two areas from which the earliest northern collections of *I. scapularis* were made in the 1970s, one in coastal New England and the other in northwestern Wisconsin (Spielman et al. 1985). The decrease in density away from these areas and the highest correlation between sites at the smallest spatial distance (300 km) are consistent with expansion from focal areas. Kitron and Kazmierczak (1997) found that the distance of peak spatial autocorrelation for tick endemicity was 160 km in Wisconsin, and Glavanakov et al. (2001) found that spatial autocorrelation of incidence rates in New York state were significant and positive up to 120 km. The minimum separation between our sampling sites did not allow for detection of spatial patterns at distances smaller than 300 km. Increasing the density of sampling sites scheduled in future years of this project will allow for detection of finer spatial structure and a more clear delineation of the boundaries of these clusters.

I. scapularis nymphs were rarely collected south of the 39th parallel (Fig. 4) (21 nymphal *I. scapularis* collected in 223,400 km of drag sampling versus 1,384 nymphs collected in 207,600 km of drag sampling north of this latitude). Because of the uniform pattern of zero or very low nymphal collections in most sites, low-density clusters were only detected when larger distances were used in the calculation of *Gi**. Although *I. scapularis* populations are present in the southern states, nearly all of the collections reported in previous studies of host-seeking ticks were of adults (Dennis et al. 1998). Questing nymphal *I. scapularis* are difficult to collect by drag sampling in the south (Piesman 2002) and are very rarely found on people (Felz et al. 1996).

Patterns of *I. scapularis* density in the northeast were generally consistent with those found in previous studies. The absence of *I. scapularis* in the Pennsylvania samples was likely because of the highly fragmented distribution of ticks in that state (Dennis et al. 1998), the lack of sampling sites located in the southeastern fringe classified as "intermediate risk" by Daniels et al. (1993), and the relatively high altitude (between 378 and 680 m) of the three randomly selected sites. High (between 8.2 and 48 nymphs per 1,000 m^2) nymphal densities were collected in Westchester County, in southern New York state, an

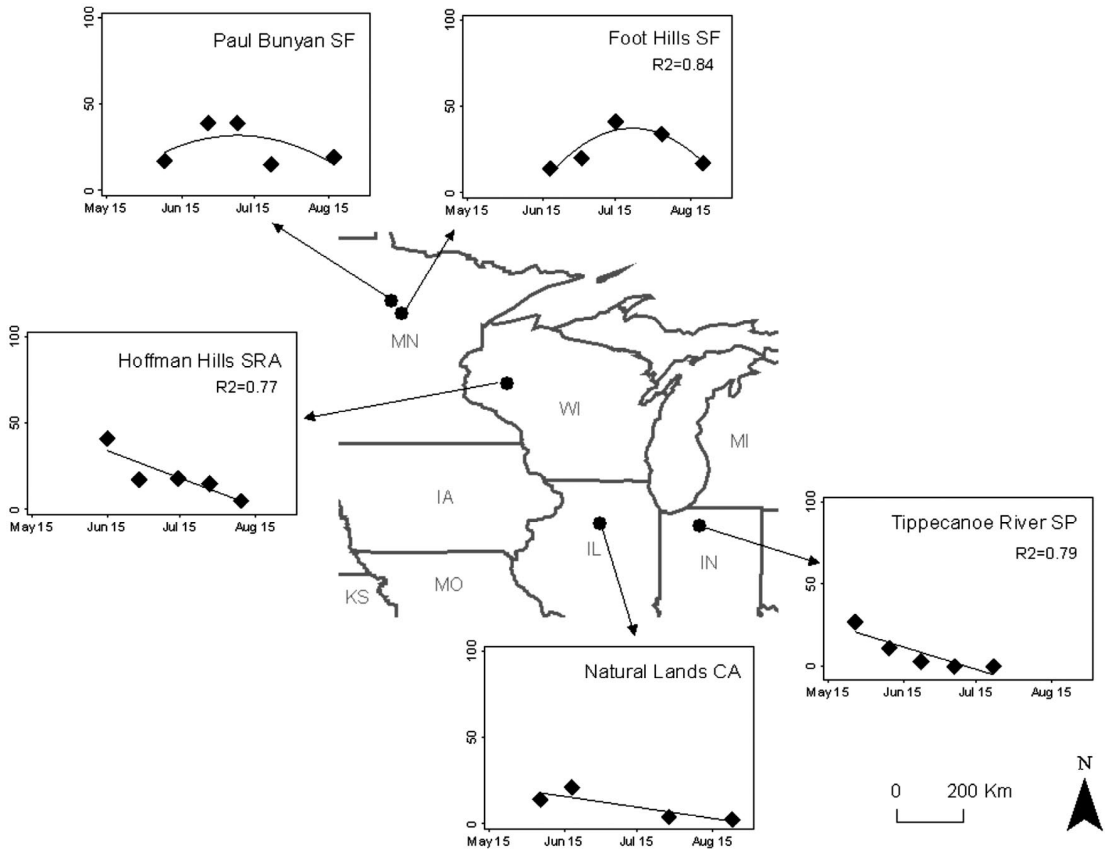

Fig. 5B. Density of *I. scapularis* nymphs per 1,000 m^2 (*y*-axis) collected in each visit (date in *x*-axis) from upper Midwest sampling sites. The best fitting curve (linear or quadratic) is shown for each collection. R^2 values are shown for those curves that had a significant fit ($P < 0.05$).

area where Lyme disease has been endemic since 1982 (Williams et al. 1986). The two northern sites in New York showed low (fewer than 0.7 nymphs per 1,000 m^2) and high densities of nymphs, whereas the more westerly site was negative, matching previously reported patterns of a decline in risk from east to west in New York state (White et al. 1991, Daniels et al. 1993, Dennis et al. 1998, Glavanakov et al. 2001). In Maine, nymphal densities were high in Crescent Beach State Park, which is in a known endemic coastal area (Holman et al. 2004). Nymphs were absent in the northern site in Maine, which is consistent with the absence of tick submissions reported by Rand et al. (1991), but not with its classification as "established" in Penobscot County by Dennis et al. (1998). The high density site in Maryland was located in the upper eastern coastal plains, where *I. scapularis* is well established (Amerasinghe et al. 1992, Glass et al. 1994, Dennis et al. 1998).

Moderate (between 0.7 and 8.2 nymphs per 1,000 m^2) nymphal densities were observed in the inland site sampled in Worcester County, Massachusetts (80 km from the coast). This is consistent with the classification as "reported" by Dennis et al. (1998) and as intermediate risk by Daniels et al. (1993). Intermediate tick densities were also found in the site in Chesterfield County, Virginia (60 km from the coast). Little is known about the occurrence of *I. scapularis* away from coastal areas in Virginia (Sonenshine et al. 1995). Future intensive sampling may help clarify the pattern and extent of *I. scapularis* inland expansion in these areas.

In the upper Midwest cluster, all four sites in Wisconsin were positive for *I. scapularis.* This was in accordance with previous studies (Guerra et al. 2002), which found positive sites in western and southern Wisconsin and in some counties in the east, including Sheboygan County, where the most eastern site was located. Ticks were collected in all five Minnesota sites. *I. scapularis* had been recognized in Beltrami, Carlton, and Cass counties (Sanders and Guilfoile 2000) as well as Sherburne County (D. Neitzel, personal communication), but the collection from Kandiyohi County was the first (D. Neitzel, personal communication). The only collections of *I. scapularis* in Michigan were in Menominee County, consistent with the presence of a focus of Lyme disease there (Walker et al. 1994, 1998). Both sites in Illinois were positive, in accordance with previous reports: one in Putnam County (Cortinas et al. 2002) and the other in

Clark County (Illinois Department of Public Health 2005). *I. scapularis* were collected in Jasper and Newton counties in northwestern Indiana, consistent with reports of an established population in this area (Pinger et al. 1991, 1996).

The seasonal host-seeking phenology must be considered in the calculation of risk; risk is null when no nymphs are host seeking, irrespective of the actual density of nymphs in a site, and increases with host-seeking tick density in endemic areas (Mather et al. 1996a). Variability in host-seeking phenology related to geography, climate, or altitude has been described previously for *I. pacificus* (Eisen et al. 2003) and for *I. ricinus* (Randolph et al. 2000; Jouda et al. 2004a,b). The extensive range of this study allowed for the detection of geographic differences in nymphal *I. scapularis* host-seeking phenology. The four most northern sites sampled showed a later start in nymphal host seeking, which permitted the capture of the full seasonal curve. A quadratic fit best represented the expected change in host-seeking phenology in these sites and allowed a more accurate identification of the time of peak nymphal activity and more accurate density estimation. In contrast, a decreasing linear trend was observed in the rest of the sites, where our sampling missed the early rise in host-seeking nymphal activity.

Mean number of *I. scapularis* over the five transects and several sampling dates was used as an estimate of density at each site. Averaging over five transects accounted in part for the variability within each site. In terms of seasonal variation, the density estimate is expected to be more accurate in those sites where the full phenological curve was captured, although it may not be as accurate for those with declining densities, where the full curve was not captured. Peak nymphal activity in the northeast is in early June (Fish 1993). Because most collections started in early June and highest nymphal densities were recorded then, it can be assumed that the complete right side of the curve was captured. Including the left part of the curve is expected to produce the same mean density value, so bias may not be significant. In the future, however, sampling will be started earlier in the season in an effort to capture the full phenological curve and estimate density by integrating values over the entire season. The good fit to a quadratic curve for Verona Beach State Park density data (visited six times) and acceptable fit for Foot Hills State Forest (visited five times) suggest that five or six visits produce a fairly accurate density estimate.

A preliminary estimate of *I. scapularis* host-seeking nymphal density was generated for its known range in the United States during the first year of this project. Given the low number of sampling points that were distributed over an extensive geographic area, there were necessarily "gaps" in the model. In future years, we will sample additional areas to improve the resolution of the clusters by more clearly defining the levels of risk within the known clusters and more accurately defining the boundary areas of the clusters, into which the range of *I. scapularis* might potentially expand.

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