

Enhancement of Risk for Lyme Disease by Landscape Connectivity, New York, New York, USA

Appendix

Screening of *Ixodes scapularis* Ticks for Infection with *Borrelia burgdorferi*

Screening of nymphal *Ixodes scapularis* ticks was conducted by extracting genomic DNA and subsequent PCR by using the DNeasy Blood and Tissue Kit (QIAGEN, <https://www.qiagen.com>) or DNA-zol BD (Molecular Research Center, <https://www.mrcgene.com>) according to the manufacturers' recommendations with modifications (1). In brief, ticks were washed twice with autoclaved deionized water and homogenized in microtubes containing 400 μ L DNA-zol BD by using the extended end of an autoclaved large paper clip or a copper BB and a vibration mill (Model MM301; Retsch, <https://www.retsch.com>). The homogenates were centrifuged at $14,000 \times g$ for 10 min, and supernatant was transferred into a new microtube. After adding 3 μ L of Poly Acryl Carrier (Molecular Research Center) to the supernatant, DNA was then precipitated by using absolute ethanol. The DNA pellet was washed twice with 75% ethanol, air-dried briefly, reconstituted in 30 μ L of $1 \times$ TE buffer (10 mmol/L Tris-HCl [pH 8.0], 1 mmol/L EDTA), and stored at -20°C for further analysis.

Isolated DNA from the ticks served as templates in subsequent PCRs to screen for infection with *B. burgdorferi* by using specific primer sets for flagellin (2), the 16S rRNA (3), and outer surface protein A (4) genes. DNA isolated from *B. burgdorferi* cultures was used as a positive control, and DNA from uninfected laboratory-reared ticks was used as a negative control in all PCRs. The positive control used was DNA isolated from a *B. burgdorferi* strain 2591 (5) culture. A Taq PCR Core Kit (QIAGEN) was used for all PCRs according to the manufacturer's recommendation. A 50- μ L reaction volume was prepared with 3 μ L template DNA, 4 μ L each primer (0.1–0.5 μ mol/L), 5 μ L $10\times$ QIAGEN PCR Buffer (containing 15 mmol/L MgCl_2), 1 μ L

dNTP mixture (10 mmol/L of each dNTP), 0.25 μ L Taq DNA polymerase (1.25 U/reaction), and 32.75 μ L water.

PCR cycling conditions for flagellin were an initial reaction activation step of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The final cycle was a 5-min extension at 72°C. Cycling conditions for 16S rRNA were an initial reaction activation step of 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min and 20s. The final cycle was completed with extension for 7 min at 72°C. Cycling conditions for the outer surface protein A gene were a reaction activation step at 94°C for 10 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 45s, and extension at 72°C for 2 min. The final cycle was completed with extension for 7 min at 72°C.

All PCRs were performed with Veriti or the GeneAmp PCR System 9700 (Applied Biosystems, <https://www.thermofisher.com/us/en/home/brands/applied-biosystems.html>). PCR-amplified products were subjected to electrophoresis on 0.6%–1.2% agarose gels, stained with ethidium bromide, visualized under UV light, and documented by using the GelDoc System (UVP, <https://www.uvp.com>). A randomly selected subsample of the PCR products was selected from the positive control and the sample for sequencing to ensure the product corresponded to the correct genes. When ≥ 2 of the 3 genetic markers for *B. burgdorferi* were identified in the sample, the sample was identified as being positive.

Landscape Connectivity Metrics

Circuitscape (<https://circuitscape.org>) simulates electrical currents through circuit networks that represent potential animal movement across habitat types with varying levels of resistance. The 2 inputs into the Circuitscape program are a shapefile that defines the nodes, or the sources of the populations, and a raster file of the landscape where land cover types and environmental attributes have different levels of resistance for animal movement. We included the 13 parks we sampled on Staten Island, NY, USA, as nodes. We parameterized the raster layer for input into Circuitscape according to the resistance values each land cover class in the matrix imposes on white-tailed deer movement. The assigned resistance values were based on studies of deer movement (6–8) and deer gene flow (9–11) (Appendix Table 2). We applied Linkage Mapper (12), which uses parameters from Circuitscape, to identify the least cost paths between

neighboring core areas. The least cost paths network was then read into the Centrality Mapper (13) program to assign each link between nodes a resistance that equals the cost-weighted distance of the corresponding least cost path (13). Centrality Mapper then applies 1 amp of current into a pair of nodes, iterating through each possible pair of nodes, to calculate the sum of the current across all nodes and connections. From the Centrality Mapper analysis, we obtained a flow centrality score for each sampled park on Staten Island that was used as a covariate in the tick abundance and infection prevalence models.

Circuitscape Analysis for White-Footed Mice (*Peromyscus leucopus*)

We ran Circuitscape and used white-footed mice resistance values (Appendix Table 5) to examine the relationship between flow centrality according to deer and mice resistance on nymphal infection prevalence. There was no major relationship between mice centrality and the nymphal infection prevalence although the major relationship between deer centrality and nymphal infection prevalence remained (Appendix Table 6). This finding might be caused by the fact that the resistance values used for deer represent those that would be applied to other reservoir hosts for *Borrelia burgdorferi* that require similar habitats to that of deer.

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Appendix Table 1. Park areas and transect lengths sampled for *Ixodes scapularis* ticks, New York, NY, USA

Park area, ha	Transect coverage, m ²
25–78	550
79–111	700
112–257	850
258–380	1,000

Appendix Table 2. Circuitscape resistance values for parks sampled for *Ixodes scapularis* ticks, New York, NY, USA*

Land cover class	Resistance value
Tree canopy	1
Grassland/shrub	1
Bare soil	30
Water	100
Paved surfaces	100
Roads/railroads	300
Buildings	1,000

*Resistance values for deer were assigned to each land cover class used in Circuitscape. Resistance values were derived from Girardet et al. (6).

Appendix Table 3. Buffer spatial scales used to model the abundance of *Ixodes scapularis* nymphs, New York, NY, USA*

Land cover class	Coefficient estimate	p value	Buffer size, m	ΔAIC
Tree canopy	0.80	<0.001	100	–
	0.65	<0.001	200	11.77
	0.48	<0.001	300	19.82
	0.38	<0.001	400	23.44
	0.31	0.005	500	25.36
Grassland/shrub	–0.27	0.013	100	–
	–0.14	0.189	200	2.62
	–0.08	0.476	300	3.43
	–0.07	0.532	500	3.46
	–0.06	0.572	400	3.55
Bare soil	–1.52	<0.001	300	–
	–0.95	<0.001	200	15.17
	–0.79	<0.001	100	17.28
	–0.87	<0.001	400	17.97
	–0.62	<0.001	500	30.45
Water	–0.55	<0.001	100	–
	–0.46	<0.001	200	3.35
	–0.63	<0.001	500	4.57
	–0.52	<0.001	400	5.35
	–0.41	<0.001	300	7.56
Impervious surfaces	–0.28	0.009	100	–
	–0.22	0.046	200	2.56
	–0.15	0.165	300	4.66
	–0.11	0.325	400	5.83
	–0.05	0.607	500	6.70

*Shown are results from the univariate negative binomial generalized linear models used to assess the best spatial scale for the land cover buffers to describe tick abundance. The buffer sizes were assessed through AIC comparison of all 5 buffer sizes for each land cover class. The buffer scale that resulted in the lowest AIC is in bold. The ΔAIC column shows the difference in AIC values between the model with the lowest AIC (indicated by –) and all other nested models within 2 AIC. AIC, Akaike's Information Criterion.

Appendix Table 4. Summary of the 4 best-fitting models for *Ixodes scapularis* tick abundance, New York, NY, USA*

Model ID	Variable	Coefficient estimate	SE	p value	AIC	Weight
124	Intercept	-3.026	0.09068	<0.001	1,092.8	0.38
	% soil, 300 m	-0.548	0.13900	<0.001		
	Centrality	0.373	0.12974	0.003		
	Tree canopy area in park	0.248	0.13349	0.06		
	% water, 100 m	-0.409	0.10618	<0.001		
	% tree canopy, 100 m	-0.466	0.09483	<0.001		
128	Intercept	-3.031	0.08984	<0.001	1,093.5	0.27
	% soil, 300 m	-0.470	0.14588	0.001		
	Centrality	0.332	0.13108	0.01		
	Tree canopy area in park	0.320	0.14294	0.02		
	% water, 100 m	-0.384	0.10673	<0.001		
	% tree canopy, 100 m	0.561	0.13576	<0.001		
122	Intercept	-3.023	0.09220	<0.001	1,093.6	0.25
	% soil, 300 m	-0.669	0.13343	<0.001		
	Centrality	0.498	0.08924	<0.001		
	% water, 100 m	-0.484	0.09741	<0.001		
	% tree canopy, 100 m	0.502	0.09588	<0.001		
	% impervious, 100 m	0.153	0.12415	0.217		
126	Intercept	-3.023	0.09214	<0.001	1,095.6	0.09
	% soil, 300 m	-0.662	0.13857	<0.001		
	Centrality	0.499	0.09018	<0.001		
	% water, 100 m	-0.483	0.09737	<0.001		
	% tree canopy, 100 m	0.526	0.13487	<0.001		
	% impervious, 100 m	0.041	0.11724	0.724		

*Shown are best selected models for *I. scapularis* tick abundance response variable (based on lowest AIC values and cumulative AIC weight). Modeling was performed by using generalized linear models with standardized explanatory variables. All covariates had variable inflation factor multicollinearity scores <4. Descriptive statistics include coefficient estimates, p values represent significance of each predictor variable in the model, the model's AIC score, and model weight. All models make up a cumulative AIC weight >0.95 and were used in the averaged model. The total AIC weight was 0.99. AIC, Akaike's Information Criterion; ID, identification.

Appendix Table 5. Circuitscape resistance values for white-footed mice (*Peromyscus leucopus*)*

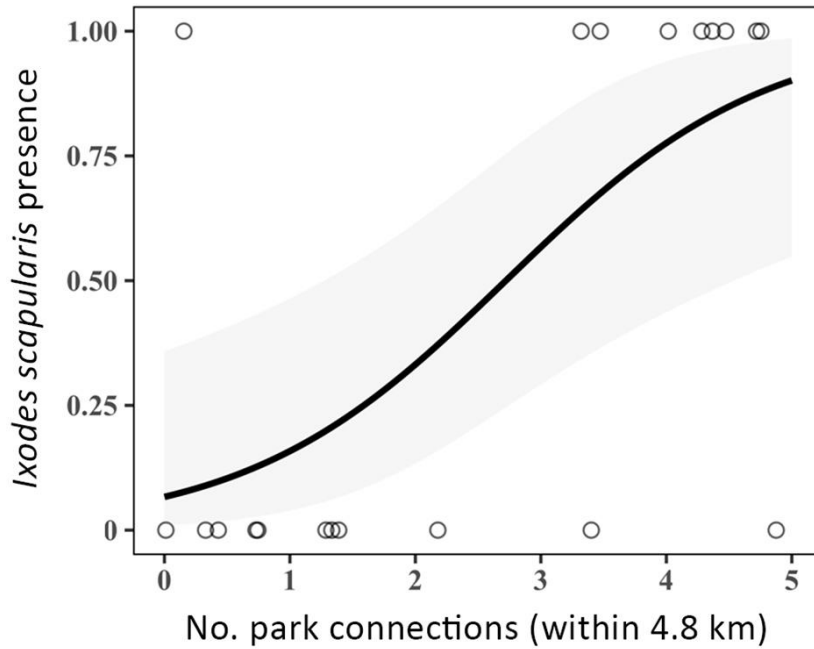
Land cover class	Resistance value
Tree canopy	1
Grassland/shrub	300
Bare soil	600
Water	1,000
Paved surfaces	900
Roads/railroads	800
Buildings	900

*Resistance values were assigned to each land cover class used in Circuitscape (<https://circuitscape.org>). Resistance values were derived from multiple sources (14–16).

Appendix Table 6. Model results for nymphal infection prevalence on Staten Island, New York, USA*

Predictor	Coefficient estimate	SE	p value
Mice centrality	-0.1929	0.1432	0.1779
Deer centrality	0.4156	0.1484	0.0051

*Shown are generalized linear model results for deer and mice centrality as predictors of nymphal infection prevalence. Deer centrality remained significant and mice centrality was not found to be a significant predictor.



Appendix Figure. Number of park connections within 4.8 km of each park showing a strong correlation with presence of *Ixodes scapularis* ticks at sampled sites, New York, NY, USA. Shown are results of the binomial generalized linear model ($p = 0.005$). SE (± 0.6787) is indicated in gray. The coefficient estimate is 1.8912.