

# Supporting Information

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## SI Materials and Methods

**DNA Extraction and PCR.** Ticks were incubated for 2 h in 5  $\mu$ L of 1.4M  $\text{NH}_4\text{OH}$  at room temperature and then crushed with a pipette tip. 95  $\mu$ L of water was added followed by a second incubation at 95  $^\circ\text{C}$  for 30 min. Material was centrifuged to separate tick debris from DNA solution, and the supernatant was transferred to clean vials containing 1  $\mu$ L of 0.1 M EDTA and stored at  $-20^\circ\text{C}$ . Real-time PCR to detect *B. burgdorferi* was performed using described primers and probe (1); PCR primer sequences were: forward (5'-GCTGTAAACGATGCACACT-TGGT) and reverse (5'-GGCGGCACACTTAACACGTTAG) and a dye-labeled probe specific to *B. burgdorferi* was (5'-6FAM-TTCGGTACTAACTTTTAGTTA-MGBNFQ), where 6FAM is the dye, and MGBNFQ is a minor groove-binding nonfluorescent quencher. The primers and probe were at concentrations of 900 and 200 nM, respectively. All reactions were done using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) with the following PCR conditions: 50  $^\circ\text{C}$  for 2 min, 95  $^\circ\text{C}$  for 10 min, and then 45–50 cycles of 95  $^\circ\text{C}$  for 15 s alternating with 63  $^\circ\text{C}$  for 60 s. The control standard for the real-time PCR assay was *B. burgdorferi* strain B31 genomic DNA.

**Multilocus Sequence Typing.** Multilocus sequence typing was performed by sequencing PCR-amplified MLST gene fragments.

Ten  $\mu$ L aliquots of each tick extract were diluted to an approximate concentration of 10 *rrs* gene copies/ $\mu$ L according to the real-time quantitative PCR screening assay. Three to five microliters of diluted extract were used in separate reactions to amplify each of eight MLST gene loci as described in ref. 2. PCR products were visualized on a 1% agarose gel, then purified and sequenced in both the forward and reverse directions using the same primers as used for PCR. Forward and reverse sequence chromatograms were visually examined, assembled and trimmed using ContigExpress, part of the VectorNTI Advance version 10 software suite (Invitrogen).

**Spatial Dependence Calculations.** Moran's *I* ranges from  $-1$  to  $1$  with a value of  $0$  indicating no spatial autocorrelation, positive values indicating positive spatial clustering, and negative values indicating negative autocorrelation. Moran's *I* was calculated for 10 equally sized distance classes, with the smallest distance class given an upper bound of 200 km, approximately the maximum distance between any site and its nearest neighbor, and the largest distance class given an upper bound of 2,000 km or the approximate scale of the entire study area. To test for significance, observed values of Moran's *I* were compared with the corresponding frequency distributions obtained when 10,000 random permutations of the data were performed.

1. Tsao JI, et al. (2004) An ecological approach to preventing human infection: Vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proc Natl Acad Sci USA* 101:18159–18164.

2. Margos G, et al. (2008) MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. *Proc Natl Acad Sci USA* 105:8730–8735.

Table S1. Samples used in this study

Strain	ST	Collection site	State	Region	Year	Latitude	Longitude
16812	1	Crescent Beach SP	ME	NE	2004	43° 33' 52.92" N	70° 14' 18.24" W
1989503	1	Presque Isle SP	PA	NE	2005	42° 9' 55.45" N	80° 6' 38.17" W
22906	3	Fordham University	NY	NE	2004	41° 7' 28.9" N	73° 43' 55.53" W
22925	3	Fordham University	NY	NE	2004	41° 7' 28.9" N	73° 43' 55.53" W
498801	3	Connetquot River SP	NY	NE	2005	40° 45' 52.82" N	73° 9' 1.21" W
501604	3	Connetquot River SP	NY	NE	2005	40° 45' 52.82" N	73° 9' 1.21" W
503205	3	Connetquot River SP	NY	NE	2005	40° 45' 52.53" N	73° 9' 35.68" W
990504	3	Fordham University	NY	NE	2004	41° 7' 39.51" N	73° 43' 50.35" W
16901	4	Crescent Beach SP	ME	NE	2004	43° 33' 36.48" N	70° 14' 5.88" W
23009	7	Fordham University	NY	NE	2004	41° 7' 43.92" N	73° 43' 56.23" W
440607	7	Connetquot River SP	NY	NE	2005	40° 45' 0.4" N	73° 9' 11.27" W
552301	7	Devils Hopyard SP	CT	NE	2005	41° 28' 40.27" N	72° 20' 4.51" W
553413	7	Connetquot River SP	NY	NE	2005	40° 45' 0.4" N	73° 9' 11.27" W
990503	8	Fordham University	NY	NE	2004	41° 7' 39.51" N	73° 43' 50.35" W
15506	9	Crescent Beach SP	ME	NE	2004	43° 34' 29.52" N	70° 14' 47.76" W
114311	11	Elk Neck SP	MD	NE	2004	39° 29' 1.74" N	75° 58' 18.66" W
519508	12	Van Buren SP	MI	MW	2005	42° 19' 48.84" N	86° 18' 33.85" W
15912	14	Crescent Beach SP	ME	NE	2004	43° 33' 36.48" N	70° 14' 5.88" W
113616	14	Elk Neck SP	MD	NE	2004	39° 29' 1.74" N	75° 58' 18.66" W
15702	18	Crescent Beach SP	ME	NE	2004	43° 33' 54.42" N	70° 14' 14.22" W
16201	18	Crescent Beach SP	ME	NE	2004	43° 33' 54.42" N	70° 14' 14.22" W
16305	18	Crescent Beach SP	ME	NE	2004	43° 33' 52.92" N	70° 14' 18.24" W
16909	18	Crescent Beach SP	ME	NE	2004	43° 33' 36.48" N	70° 14' 5.88" W
17311	18	Crescent Beach SP	ME	NE	2004	43° 33' 52.92" N	70° 14' 18.24" W
24602	18	Fordham University	NY	NE	2004	41° 7' 31.38" N	73° 43' 54.07" W
15903	19	Crescent Beach SP	ME	NE	2004	43° 33' 36.48" N	70° 14' 5.88" W
47703	29	Foothills SF	MN	MW	2004	46° 49' 0.9" N	94° 35' 58.44" W
47803	29	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 3" W
50901	29	Foothills SF	MN	MW	2004	46° 48' 49.44" N	94° 35' 52.86" W
2080802	29	Castle Rock SP	IL	MW	2005	41° 59' 9.13" N	89° 21' 38.97" W
2085801	29	Banning SP	MN	MW	2005	46° 10' 18.48" N	92° 50' 43.38" W
51405	30	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 5.46" W
48102	31	Paul Bunyan SF	MN	MW	2004	47° 9' 13.38" N	94° 52' 30.12" W
50302	31	Paul Bunyan SF	MN	MW	2004	47° 9' 13.38" N	94° 52' 30.12" W
519014	32	Van Buren SP	MI	MW	2005	42° 19' 48.84" N	86° 18' 33.85" W
519512	32	Van Buren SP	MI	MW	2005	42° 19' 48.84" N	86° 18' 33.85" W
519519	32	Van Buren SP	MI	MW	2005	42° 19' 48.84" N	86° 18' 33.85" W
519612	32	Van Buren SP	MI	MW	2005	42° 19' 50.46" N	86° 18' 35.83" W
2207117	32	Wildcat Mountain SP	WI	MW	2005	43° 42' 22.28" N	90° 33' 57.74" W
2215505	32	Mirror Lake SP	WI	MW	2005	43° 34' 1.92" N	89° 48' 4.95" W
24209	34	Fordham University	NY	NE	2004	41° 7' 28.9" N	73° 43' 55.53" W
500203	36	Connetquot River SP	NY	NE	2005	40° 45' 18.18" N	73° 9' 5.72" W
23509	37	Fordham University	NY	NE	2004	41° 7' 28.9" N	73° 43' 55.53" W
501442	37	Connetquot River SP	NY	NE	2005	40° 45' 0.4" N	73° 9' 11.27" W
501614	37	Connetquot River SP	NY	NE	2005	40° 45' 52.82" N	73° 9' 1.21" W
15905	38	Crescent Beach SP	ME	NE	2004	43° 33' 36.48" N	70° 14' 5.88" W
119909	38	Gifford Pinchot SP	PA	NE	2005	40° 4' 32.05" N	76° 54' 53.92" W
516113	38	Devils Hopyard SP	CT	NE	2005	41° 27' 44.25" N	72° 20' 50.82" W
2007515	38	Connetquot River SP	NY	NE	2005	40° 45' 52.53" N	73° 9' 35.68" W
1472505	39	Hartman Creek SP	WI	MW	2004	44° 19' 32.82" N	89° 18' 42.48" W
51108	43	Paul Bunyan SF	MN	MW	2004	47° 10' 17.94" N	94° 52' 42.06" W
51803	43	Foothills SF	MN	MW	2004	46° 48' 49.44" N	94° 35' 52.86" W
50103	44	Foothills SF	MN	MW	2004	46° 48' 49.44" N	94° 35' 52.86" W
553701	45	Connetquot River SP	NY	NE	2005	40° 45' 18.18" N	73° 9' 5.72" W
47902	46	Foothills SF	MN	MW	2004	46° 49' 0.96" N	94° 35' 57.18" W
48803	46	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 5.46" W
50306	46	Paul Bunyan SF	MN	MW	2004	47° 9' 13.38" N	94° 52' 30.12" W
50609	46	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 3" W
1476702	46	Hartman Creek SP	WI	MW	2004	44° 19' 11.1" N	89° 13' 27.54" W
47601	47	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 5.46" W
48802	47	Foothills SF	MN	MW	2004	46° 49' 4.32" N	94° 36' 5.46" W
1457401	48	Jay Cooke SP	MN	MW	2004	46° 39' 17.52" N	92° 20' 59.94" W
1468503	48	Mirror Lake SP	WI	MW	2004	43° 33' 34.56" N	89° 49' 3.78" W
2228004	48	Van Buren SP	MI	MW	2005	42° 20' 16.2" N	86° 18' 13.25" W

Strain	ST	Collection site	State	Region	Year	Latitude	Longitude
1468904	49	Hartman Creek SP	WI	MW	2004	44° 19' 28.8" N	89° 13' 42" W
501605	50	Connetquot River SP	NY	NE	2005	40° 45' 52.82" N	73° 9' 1.21" W
424404	51	Banning SP	MN	MW	2005	46° 10' 18.48" N	92° 50' 43.38" W
2127701	52	Wildcat Mountain SP	WI	MW	2005	43° 42' 19.05" N	90° 33' 49.2" W
2207601	53	Black River SF	WI	MW	2005	44° 14' 29.33" N	90° 34' 52.68" W
2208804	53	Brunet Island SP	WI	MW	2005	45° 10' 48.61" N	91° 9' 35.27" W
501427	54	Connetquot River SP	NY	NE	2005	40° 45' 0.4" N	73° 9' 11.27" W
424401	55	Banning SP	MN	MW	2005	46° 10' 18.48" N	92° 50' 43.38" W
2206617	55	Brunet Island SP	WI	MW	2005	45° 10' 48.61" N	91° 9' 35.27" W
1469205	56	Hartman Creek SP	WI	MW	2004	44° 19' 9.42" N	89° 13' 31.56" W
2250201	57	Parker Dam SP	PA	NE	2005	41° 12' 40.98" N	78° 30' 2.99" W
114212	58	Elk Neck SP	MD	NE	2004	39° 28' 40.02" N	75° 58' 48.9" W
1988101	59	Presque Isle SP	PA	NE	2005	42° 9' 18.17" N	80° 6' 22.27" W
2251413	59	Gifford Pinchot SP	PA	NE	2005	40° 4' 32.05" N	76° 54' 53.92" W

SP, State Park; SF, State Forest; NE, Northeast; MW, Midwest.

Table S2. Sequence types

ST	Allelic profile							
	<i>nifS</i>	<i>clpA</i>	<i>pyrG</i>	<i>recG</i>	<i>clpX</i>	<i>pepX</i>	<i>uvrA</i>	<i>rplB</i>
1	1	1	1	1	1	1	1	1
3	1	4	1	6	1	1	7	1
4	1	8	4	6	1	1	7	1
7	5	6	1	7	1	1	8	1
8	4	5	5	5	5	5	6	1
9	4	10	1	6	5	6	6	1
11	5	5	6	1	7	1	9	4
12	2	3	3	4	3	4	4	4
14	1	9	1	6	1	7	10	1
18	6	7	1	5	6	1	5	5
19	3	4	3	3	4	3	3	3
29	1	18	2	15	12	11	2	1
30	5	19	2	1	1	1	10	1
31	3	20	3	3	4	3	3	3
32	1	8	4	16	1	1	7	1
34	1	8	1	6	1	7	10	1
36	4	10	1	15	5	6	6	1
37	12	7	1	5	6	1	5	5
38	15	21	1	18	1	8	7	4
39	1	12	1	16	2	2	4	13
43	3	4	3	21	4	3	3	6
44	3	4	3	3	4	19	3	3
45	5	6	1	7	1	1	8	12
46	12	7	2	5	6	1	5	5
47	12	7	6	5	6	1	5	5
48	1	8	2	6	1	14	10	1
49	1	8	2	6	1	15	10	1
50	1	8	1	6	1	17	10	1
51	15	8	12	20	1	8	7	1
52	1	12	1	6	1	7	10	1
53	1	12	1	6	1	13	10	1
54	16	15	1	18	1	4	20	4
55	17	23	2	1	1	20	10	1
56	4	24	11	19	14	18	12	1
57	3	25	3	21	4	16	2	1
58	5	6	1	7	1	1	1	1
59	5	6	1	7	1	1	19	1

**Table S3. MLST primers used in this study**

Gene	Orientation	Primer designation	5' → 3' sequence
<i>nifS</i>	Forward	nifSF1–23	ATGGATTTCAAACAAATAAAAAG
	Reverse	nifSr719	GTTGGAGCAAGCATTTTATG
<i>clpA</i>	Forward	clpAF1240	GATAGATTCTCCAGACAAAG
	Reverse	clpAr2104	CAAAAAAAAAACATCAAATTTCTATCTC
<i>pyrG</i>	Forward	pyrGF448	GATATGGAAAATATTTTATTATTG
	Reverse	pyrGr1190	CAAACATTACGAGCAAATTC
<i>recG</i>	Forward	recGF917	CTTTAATTGAAGCTGGATATC
	Reverse	recGr1694	GAAAGTCCAAAACGCTCAG
<i>clpX</i>	Forward	clpXF403	AATGTGCCATTGCAATAGC
	Reverse	clpXr1124	TTAAGAAGACCCCTAAAATAG
<i>pepX</i>	Forward	pepXF449	TTATTCCAAACCTTGCAATCC
	Reverse	pepXr1172	GTTCCAATGTCAATAGTTTC
<i>uvrA</i>	Forward	uvrAF1408	GAAATTTTAAAGGAAATAAAAGTAG
	Reverse	uvrAr2111	CCTATTGGTTTTTGATTATTG
<i>rplB</i>	Forward	rplBF40	CGCTATAAGACGACTTTATC
	Reverse	rplBr834	CTATTTATTTCTCTTTAATAATAAA